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with international participation**

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ABSTRACT BOOK



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Artificial intelligence in laboratory medicine

PCS-1

From text to tables: using large language models-generated structured outputs to answer scientific questions

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Laboratory medicine generates vast amounts of heterogeneous data – numerical results, instrument readouts, diagnostic images, clinical free-text reports, and a growing body of scientific literature – much of which remains underutilized because transforming it into structured, analyzable form is laborious. Large language models (LLMs) have emerged as versatile tools capable of processing text, images, and multimodal inputs simultaneously, able to return answers as machine-readable structured data rather than free-form prose. This capability opens broad opportunities across laboratory disciplines: from automated image interpretation to systematic evidence extraction from patient records and published research.

From images to answers: multimodal LLMs as classifiers

The first project demonstrates structured LLM outputs applied to visual data in medical microbiology – a model transferable to any laboratory discipline that relies on image-based readouts. Images of agar plates from routine requests were segmented using custom open-source software and submitted with structured prompts to multimodal LLMs (Gemma 4 E4b, Qwen 3.5 4b, etc.) served locally *via* Ollama. Without task-specific training, models achieved accuracies up to 0.975. Critically, models were instructed to return structured outputs – thus transforming a narrative model response into a tabular data point that can be statistically evaluated. The same approach could potentially be applied in laboratory medicine more broadly: automated reading of lateral flow assays or microscopy images without requiring custom-trained computer vision models.

From literature to knowledge: LLM-assisted text mining

The second project applies structured LLM outputs to large corpora of published text – again with direct parallels beyond microbiology. The immediate goal is to classify bacterial species by human pathogenicity: with over 28,000 described species and numbers growing at roughly 28% in recent years, manual curation is no longer feasible. The proposed pipeline retrieves literature *via* PubMed, extracts full text and stores data in a relational database. LLMs then respond to a standardized questionnaire – whether a pathogen was isolated from sterile sites, whether isolation was repeated, whether clinical harm was documented – with answers constrained to structured schemas. The resulting tables are directly amenable to statistical analysis. In laboratory medicine, analogous pipelines could mine patient discharge summaries, clinical free-text findings for diagnostic patterns, or systematically review the literature on biomarker performance – tasks currently requiring months of manual expert effort.

Conclusion

Together, these projects illustrate a generalizable workflow: define a scientific question, encode it as a structured prompt schema, submit inputs – images, abstracts, full texts, or free-text clinical records – to an LLM, and harvest machine-readable answers suitable for conventional analysis. The key shift in mindset is to treat LLMs not as chatbots, but as intelligent data-extraction engines. Laboratory professionals are uniquely positioned to exploit this paradigm: their deep domain knowledge enables question formulation, validation against ground-truth datasets, and critical appraisal of model outputs. Whether the goal is reading an agar plate, classifying a pathogen from the literature, extracting a diagnosis from a clinical narrative, or synthesizing evidence across thousands of publications, structured LLM outputs offer a scalable path from unstructured data to scientific insight.

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PCS-2**The 21st century biochemist: how practical AI applications improve and simplify laboratory work**

Mario Štefanović

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Artificial intelligence (AI) is becoming an integral part of modern laboratory medicine; however, its application does not need to be limited to large research centers nor require advanced programming skills. This lecture will demonstrate how laboratory professionals can utilize modern AI tools for advanced data analysis, automation of analytical workflows, and more efficient management of laboratory processes.

Through practical examples, the use of tools such as Python, Jupyter Notebook, and Google Colab will be demonstrated for processing large laboratory datasets, performing reproducible analyses, conducting statistical evaluations, and developing simple solutions tailored to the needs of individual laboratories. Examples of using generative artificial intelligence to create analytical scripts without prior programming knowledge will also be demonstrated, enabling laboratory professionals to independently develop tools for data processing and the automation of routine tasks.

The lecture will further illustrate how platforms such as Power BI can be used to create interactive dashboards for monitoring laboratory performance, process quality, trends in laboratory indicators, and key performance metrics. Particular emphasis will be placed on the ability of AI to transform laboratory data into actionable information that supports decision-making, quality improvement, and process optimization.

The aim of this lecture is to demonstrate how biochemists can extend their professional capabilities far beyond traditional laboratory routines by using modern AI tools and, without formal programming education, apply advanced methods of data analysis, automation, and reporting in everyday laboratory practice.

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PCS-3

Large language models-based clinical decision support

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Laboratory medicine increasingly supports complex clinical decision-making, but interpretation of laboratory results often requires specialist knowledge and integration of multiple data points over time. Anemia diagnostics are a representative example: laboratory results need to be interpreted in relation to previous measurements, inflammation, iron status, vitamin deficiencies, kidney function and clinical context. We hypothesise that large-language models can support clinical decision-making by translating laboratory results into structured, explainable and clinically useful interpretative comments. The aim of this work is to develop and evaluate an artificial intelligence supported framework for anemia interpretation, as a first step towards broader clinical decision support in laboratory medicine.

The anemia pathway will be used as the primary use case. Existing laboratory consultations and the underlying diagnostic workflow will be analyzed to describe how clinical chemists reason from laboratory patterns towards interpretation and advice. Based on this framework, language-model based draft comments will be generated and compared with expert-written interpretations. Technical validation will be performed by clinical chemistry experts, who will rate model output for medical correctness, completeness, consistency, clarity, safety and clinical usefulness. In a subsequent implementation study, the system will be evaluated in the clinical workflow, including its integration into the electronic health record, effect on consultation workflow, user acceptance and perceived added value for clinicians.

We show the development pathway from current expert-based anemia interpretation towards model-supported draft consultation. We demonstrate a validated prototype, an expert-rating framework for technical validation and an implementation design for evaluating clinical usefulness in practice.

Large-language models may provide a scalable way to support interpretation of complex laboratory diagnostics. Starting with anemia allows technical validation and clinical implementation to be studied in a high-volume, clinically relevant workflow before extension to other diagnostic domains.

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PCS-4

False discovery rate corrections in routine laboratory diagnostics and biomarker discoveryIvana Marković^{1,2}, Željko Debeljak^{1,2}¹Clinical Institute for Laboratory Diagnostics, University Hospital Centre Osijek, Osijek, Croatia²Faculty of Medicine Osijek, JJ Strossmayer University of Osijek, Osijek, Croatia

In contemporary laboratory medicine, the development of new analytical methods and multiparametric approaches has accelerated the discovery of novel biomarkers and increased the demand for measuring numerous laboratory parameters. When many laboratory results are interpreted simultaneously, each value compared with a reference interval or a clinical cut-off can be viewed as a form of hypothesis testing, leading to an implicit decision: normal or abnormal. A conventional P-value is usually interpreted in the context of a single test. For example, a threshold of $P < 0.05$ means that, if no true difference or association exists, a statistically significant result may still occur in about 5% of repeated tests by chance alone. However, as the number of simultaneously evaluated laboratory results increases, so does the probability that at least one abnormal finding will arise randomly due to biological variation, preanalytical factors, or analytical measurement uncertainty. In other words, when 100 or 1000 biomarkers are tested at the same time, the risk of false significant findings accumulates. Such findings may misdirect diagnostics, delay or distort clinical interpretation, increase costs, and, in biomarker discovery, lead to incorrect conclusions and unnecessary validation studies.

To reduce the number of false-positive results, methods for false discovery rate (FDR) correction are used. These refer to the expected proportion of false-positive findings among all statistically significant results. Among the best-known correction approaches are the Bonferroni correction and the Benjamini-Hochberg method. The Bonferroni correction is simple: the original significance level α is divided by the number of tests, thereby reducing the risk of false-positive results. However, this approach is highly conservative and may reduce the probability of detecting truly significant findings. In contrast, the Benjamini-Hochberg method controls the proportion of false-positive results among statistically significant findings. It first ranks all P-values from smallest to largest and then compares them with adjusted thresholds that depend on the total number of tests, the rank of each P-value and the selected significance cut off. After FDR correction, some initially significant raw P-values may no longer remain significant. A limitation of this method is that a certain number of false-positive results is still tolerated, making it less suitable when even a minimal risk of false-positive interpretation is unacceptable.

The simultaneous interpretation of many routine laboratory results is not identical to formal multiple hypothesis testing based on P-values. Therefore, FDR should not be applied mechanically in routine laboratory interpretation, but rather as a conceptual warning. Its practical value is greatest in large datasets, such as autoantibody panels, allergy panels, steroid panels, and omics-based studies, including proteomics, metabolomics, and genomics, particularly in biomarker research. This lecture will be dedicated to the importance of applying FDR in multiple testing as part of the responsible interpretation of laboratory and research data.

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PCS-5

Deep learning-based classification of urinary sediment particles

Glynis Frans

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Urinalysis has progressed from manual microscopy to automated methods such as high-throughput test strip analysis, flow cytometry, and digital microscopy. Digital microscopy is particularly valuable because manual urinary sediment microscopy is labor-intensive, operator-dependent, and limited in precision and reproducibility. It is therefore well suited to artificial intelligence (AI)-based image classification, which can also help overcome common technical challenges with urinary microscopy such as low contrasts within particles, overlapping particles, and background interference. Previous studies have already applied machine learning and deep learning to urinary sediment image analysis, using both whole-slide and segmented images, manual and digital microscopy, and datasets ranging from small collections to large annotated sets.

This presentation describes the development, validation, and implementation of an AI-based tool for digital urinalysis image analysis at UZ Leuven, Belgium. A dataset covering 13 urinary sediment classes was annotated from Sysmex UD-10 digital microscope images. An EfficientNet model was trained and evaluated in three scenarios to examine the impact of data collection strategies on performance. Uncertainty calibration, Grad-CAM, and t-SNE were used to assess confidence, robustness, interpretability, and feature separation. A graphical user interface was also created for prospective laboratory evaluation. The model achieved about 97% overall test accuracy. However, performance was sensitive to dataset variability, suggesting that additional training data could further improve results. Confidence scores aligned with accuracy, and interpretability analyses showed attention to relevant image regions and clear class separation. In prospective datasets from three clinical laboratories, including UZ Leuven, top-1 and top-3 accuracies decreased to about 80% and 90%, respectively.

In summary, our findings demonstrate that deep learning can achieve robust and accurate classification of urinary sediment particles in digital microscopy images. The observed discrepancy between retrospective and prospective performance further underscores the importance of rigorous external validation and context-specific evaluation before clinical deployment. Collectively, these results highlight the considerable potential of AI to advance urinalysis, while emphasizing that successful routine implementation will require systematic validation, careful integration into laboratory workflows, and strict adherence to clinical and regulatory standards.

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PCS-6

Single-cell analysis via machine and statistical learning of mass spectrometry imagesŽeljko Debeljak, Ivana Marković

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Mass spectrometry (MS) and MS imaging (MSI) of tissues or cells, in particular, are still in their early phase of clinical development. One of main obstacles in implementation of different MS techniques is interpretation of huge amounts of data produced for each sample. In this regard, MSI represents a special case since it generates complex mass spectra for each pixel. MSI is mainly qualitative or semi-quantitative technique and, in order to provide information on cell or tissue biochemistry, it requires specific data analysis tools. Simple approach to visual representation of MSI recordings involves the representation of a single mass-to-charge (m/z) signal intensity for each pixel using color scales: if the biomolecule of interest, or appropriate m/z signal, is preselected then no special data analysis tools are required. But if the selection of the informative m/z signals, among hundreds or even thousands of signals contained in each mass spectra, is needed for tissue or cell differentiation, it requires application of advanced statistical or machine learning (ML) methods.

For research purposes, two or more tissue or cell sets are compared to find the most informative m/z signals *i.e.* m/z signals which intensities differ between the analyzed tissues or cells. Informative m/z signals may be annotated to some metabolites or proteins providing insights in tissue or cellular metabolism and proteomics. For selection of the most informative m/z signals, the use of univariate statistical tests like Kruskal-Wallis ANOVA may be appropriate. To avoid the unrealistic significance inflation, instead of intensities of numerous heavily correlated pixels, intensities averaged over the carefully selected regions of interest (ROI) are needed. Due to hundreds or even thousands of signals coming from each ROI mass spectrum, some *post hoc* significance correction is also required. Frequently, statistical significance does not properly reflect the biological significance: in these instances, it is combined with the fold change in signal intensities. And even then, results coming from univariate tests will miss interactions and nonlinear dependencies between m/z signals and their impact on differentiation of tissue or cell samples. To capture interactions and nonlinearities, multivariate statistical and ML methods combined with appropriate validation methods are needed. Among the statistical and ML classification methods for selection of m/z signals in MSI, partial least squares discriminant analysis (PLS-DA) stands out. PLS-DA may be described as a supervised learning variant of the widely used principal component analysis. If applied properly in MSI, it frequently provides reliable and interpretable metabolic or proteomic changes specific to analyzed cells or tissues their clear visual representation. These properties come with dimensionality reduction enabled by the validation accuracy based selection of principal components. In this case, m/z signals are ranked by their contribution to selected principal components: highly ranked m/z signals represent determinants for visual differentiation of tissues or cell types and their metabolic and proteomic rewiring. To show implementation, advantages and disadvantages of different statistical and ML techniques in MSI analysis, single-cell MSI research in chronic lymphocytic leukemia will be presented.

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Biochemistry and the philosophy of man

Stribor Marković

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The idea of *Vis vitalis* had roots as deep as human mysticism itself, woven into the fear and incomprehension of the enormous forces that shaped the environment of our ancestors at the edge of the cave. Even the great Claude Bernard did not always resist its allure. The world rejected René Descartes' idea of the human being as a well-oiled machine, only for the nineteenth century to usher in the beginning of the deconstruction of man into molecules that we can now literally produce in a test tube. Never had human vanity been shattered so forcefully, so much so that, even today, we have not fully admitted it.

Yet, in the deconstruction of human essence, a great deal of soul emerged. The fear that dismantling *Vis vitalis* would reduce man to the notion of an organic-chemical machine proved unnecessary. Layer by layer, biochemistry revealed the many faces of humanity: the one that comprehends, marvels, and understands complexity. Without biochemistry there is no medicine, no new drugs, no new therapeutic directions, no uncovering of ancient molecular paleontology, and no transformation in our perception of what life is and how it should be defined.

Paradoxically, the destruction of *Vis vitalis* made us more sensitive, some would even say more spiritual. I will not be quite so bold. All I want is to tell the story of the profession I chose while studying beneath the sound of thousands of shells exploding. For me, biochemistry is introspection. At seventeen, I was reading the Sarajevo textbook *Biohemija* by Professor Winterhalter-Jadrić, while my late Aunt Vava watched and said, "That madness too shall pass". She used to say that after the Second World War, people emerged from the camps carrying knowledge of chemistry, medicine, and French, so that they could rebuild a world shattered into pieces.

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Plenary lectures

PL-1

Research vs. clinical laboratory methods and regulations: understanding the key differences

Tomáš Zima

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This presentation will address the fundamental differences between research methods (immunoassays, mass spec, chromatography for research, molecular biology, in-house, *etc.*) and those employed in clinical laboratory settings (validated *in vitro* diagnostic (IVD) kits). The laboratory medicine is regulated by national directives and management quality is also implemented (*e.g.* ISO 15189). There are the similarities *e.g.* analytical process and differences in these processes across research and clinical settings and will briefly mention the regulatory requirements, analytical performance measures, and validation processes that clinical methods must undergo compared to research methods. These important issues are also important for basic research to ensure the reliability and accuracy of scientific studies so that research methods are suitable for clinical application. Examples of successful transitions from research to clinical practice will be briefly provided to illustrate the process and its impact on scientific studies.

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PL-2

Medical biochemist in the educational system

Lada Rumora

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The plenary lecture explores the pivotal role of education in shaping laboratory medicine professionals. While scientific conferences typically focus on technological innovations, diagnostic algorithms, and management systems, education remains a foundational, yet often underappreciated, component of a medical biochemist's professional identity. The lecture emphasizes that the educational process shapes not only knowledge, but also competence, ethical maturity, and professional character, all of which are crucial in a regulated healthcare profession.

Through an analysis of the integrated Medical Biochemistry program at the Faculty of Pharmacy and Biochemistry in Zagreb, the lecture dissects the multi-layered educational process. Students develop cognitive skills, critical thinking, and they learn to differentiate raw data from interpretation. Concurrently, practical laboratory competencies are built, including quality assessment, recognition of analytical interferences, and effective communication of results. Finally, the professional and ethical dimension teaches students responsibility toward patients, emphasizing that every laboratory result carries clinical and human significance.

Special attention is given to medical biochemists as educators. They embody a dual professional identity: both expert practitioners and academic instructors. Professional credibility requires continuous contact with the profession, while pedagogical competence includes moderating knowledge, encouraging critical thinking, and developing professional judgment, especially in the context of the digital generation and the availability of artificial intelligence.

Educators also serve as models of professional culture, transmitting the values and standards of the profession through their example and conduct.

The lecture further examines contemporary challenges in education, including digitalization of teaching, the integration of artificial intelligence in learning, administrative burdens, and evaluation pressures. Despite technological and institutional complexities, students remain central: their motivation, need for mentorship, and awareness of responsibility shape how the educational system develops future professionals.

At a strategic level, high-quality medical biochemistry education is directly linked to the sustainability of the profession and the quality of laboratory medicine in practice. Professional autonomy, ethical stability, and clinical competence depend on the educational system's capacity to impart knowledge, skills, and professional standards to new generations.

In conclusion, the lecture emphasizes the enduring responsibility of education in shaping the identity of the profession: education is not merely the start of a career, but the foundation that ensures professional quality, societal credibility, and the future of laboratory medicine. The future of the profession is forged in classrooms, through mentorship and the example set by educators, rather than solely through technology or algorithms.

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PL-3**Genetics and epigenetics: where are we today in translational research and clinical application**

Daria Pašalić

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Genetics and epigenetics have emerged as key drivers of innovation in modern public health, providing new insights into the biological mechanisms underlying health and disease. Advances in genomic and epigenomic research have improved the ability to identify individual and population-level risk factors for chronic non-communicable diseases, including cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders.

A central role is played by translational medicine, which bridges fundamental biomedical discoveries and their application in clinical practice and public health. The integration of genomic and epigenomic data supports more accurate risk assessment and enables the development of personalized and stratified prevention strategies tailored to specific population needs.

Epigenetics further highlights how environmental and lifestyle factors, such as nutrition, physical activity, stress, and environmental exposures, can influence gene expression throughout life. These findings emphasize the importance of a life-course approach to health and provide the foundation for precision public health, where interventions can be targeted more effectively and resources allocated more efficiently.

At the same time, the implementation of genomics and epigenetics in public health raises important ethical, legal, and social challenges, including data privacy, equitable access to emerging technologies, and the need for robust infrastructure and interdisciplinary expertise.

Overall, the integration of genomics and epigenetics has the potential to transform public health by improving disease prevention, enhancing health equity, and supporting the development of more effective and sustainable health systems worldwide.

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S1 Life-threatening bleeding: diagnosis and contemporary management

S1-1

Laboratory diagnosis in massive hemorrhage

Slavica Kvolik, Marija Milić

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In determining the need for “rapid” laboratory diagnostics, which should always be guided by the urgency of massive bleeding, it is necessary to consider two factors: the cause of the massive bleeding and the patient’s clinical condition at the time of onset and before the onset of massive bleeding. Given that no existing laboratory method is as fast as the bleeding in the most important massive bleeding, such as ruptured abdominal aortic aneurysm, severe postpartum hemorrhage, or severe trauma, it is necessary to choose a diagnostic method that will be fast, and its results will be able to be observed in the light of the current clinical picture.

The goal of laboratory diagnostics in massive bleeding is to answer several questions, such as

- What is the patient’s current coagulation status
- How extensive is the bleeding
- What are the compensatory mechanisms
- How urgent is the replacement with blood derivatives, and which of the available ones is best to apply?

The first method of “diagnosis of coagulation status” is certainly the diagnosis of the bleeding site, and the first hemostatic intervention is surgical hemostasis, wherever possible. In parallel, to avoid volume overload, sophisticated methods that identify the specific disorder are increasingly used. To determine which method will be used, it is also necessary to know the patient’s prior comorbidities and therapies. Clinicians will always make a definitive decision on a hemostatic intervention based on the results of bedside diagnostics, primarily viscoelastic coagulation tests and standard coagulation tests, along with the patient’s clinical parameters. Considering that assessing the extent of bleeding requires more than a single measurement, determining the trend of laboratory indicators of bleeding is important. When measuring once, it is necessary to include a control measurement. A goal of those measurements will be to assess the therapeutic success in the closed loop involving laboratory, transfusion services, the initially applied resuscitation methods, and the adequacy of coagulation after those interventions.

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S1-2

The role of viscoelastic testing in assessing hemostasis

Marija Milić

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Viscoelastic testing (VET) has become an important diagnostic tool in the management of patients with acute life-threatening bleeding. VETs, including thromboelastography (TEG) and rotational thromboelastometry (ROTEM), are point-of-care (POC) methods used for real-time assessment of the whole-blood coagulation. Unlike conventional laboratory coagulation assays, which are plasma-based and measure time to clot formation, VETs analyze clot initiation, propagation, strength, and clot dissolution in whole blood, thereby providing a more global overview of hemostasis. VETs have no direct equivalent among standard laboratory coagulation tests. Although some correlations exist between VET parameters and screening coagulation tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, and platelet count, these correlations are generally weak. Despite being global assays, VET methods do not fully represent physiological hemostasis. They do not incorporate the role of the endothelium, which is a key regulator of hemostatic balance, nor do they account for shear stress and flow conditions present within the blood vessel. Furthermore, VETs demonstrate limited sensitivity to mild platelet dysfunction, mild factor XIII deficiency, and von Willebrand disease-related abnormalities.

Although thromboelastography was developed several decades ago, its widespread clinical use was initially limited by technical constraints, including susceptibility to vibration and long turnaround times. Advances in technology have largely overcome these limitations through improved analyzers and the introduction of coagulation activators, resulting in faster testing. Different manufacturers employ distinct technologies to measure the viscoelastic properties of blood. Systems such as TEG, ROTEM, and ClotPro are based on pin-and-cup measurement principles, whereas newer platforms, including TEG 6s and Quantra, utilize resonance-based technologies. Differences in measurement principles, activation reagents, reported parameters, and reference intervals limit direct comparability of results and complicate the development of universally accepted clinical guidelines and transfusion algorithms. Additional challenges include the limited availability of pediatric reference intervals.

Viscoelastic testing has emerged as a valuable tool for the assessment and management of acute bleeding, particularly by enabling differentiation between surgical and coagulopathic bleeding and supporting targeted hemostatic therapy. The most robust evidence for VET-guided transfusion algorithms comes from cardiac surgery, major trauma, and liver transplantation, where implementation of VET has been associated with reduced blood product utilization and improved patient outcomes. Evidence supporting the routine use of VET in other settings, such as advanced liver disease, postpartum hemorrhage and COVID-19-associated coagulopathy, remains limited.

Current research is increasingly focused on the assessment of coagulation in patients receiving direct oral anticoagulants (DOACs), evaluation of anticoagulant reversal strategies, and validation of dedicated VET assays for DOAC detection.

Another area of growing interest is the use of VET for the assessment of hypercoagulable states and thrombotic risk, as well as sepsis-associated coagulopathy and disseminated intravascular coagulation. Another promising area of research is the integration of artificial intelligence with VET data to improve interpretation of results and support clinical decision-making.

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S1-3

Challenges in the laboratory diagnosis of acquired hemophilia

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Albeit occurs rarely, with an annual incidence ranging between 1.5 and 2.0 cases/million/year, acquired hemophilia A (AHA) is a serious and potentially a life-threatening bleeding disorder. Acquired hemophilia A develops as a result of the presence of neutralizing autoantibodies (inhibitors) against coagulation factor VIII (FVIII) in patients, regardless of gender, without a previous history of bleeding. Typically, two age peaks in AHA incidence has been observed, one associated with pregnancy, generally occurring during the postpartum period, and the other one associated with older age (> 60 years) and usually with concomitant disorders. However, AHA is still underdiagnosed or diagnosed with a delay. Therefore, the establishment of a rapid and precise diagnosis is vital for patients in order to initiate adequate therapeutic treatment as soon as possible.

An unexplained isolated prolonged activated partial thromboplastin time (aPTT) is usually the first clue of AHA and subsequent appropriate laboratory investigation should be carried on to establish and confirm the diagnosis. Various diagnostic algorithms have been suggested and according to the International recommendations on the diagnosis and treatment of AHA (Tiede A. *et al.* *Haematologica* 2020;105:1791-801) the diagnosis should be made by a stepwise approach. As isolated prolonged aPTT can result due to many different causes, the first step in the differential laboratory diagnosis of AHA is to perform an aPTT mixing study of a patient's plasma with normal plasma in a ratio 1:1. The failure to correct the prolonged aPTT in the mixing study suggests the presence of an inhibitor only, implying further investigation and determination of FVIII activity as a next step to facilitate early diagnosis. In cases when FVIII activity testing is rapidly available, aPTT mixing study could be skipped. Markedly low FVIII activity is strongly suggestive of AHA, but it has also to be emphasized that in approximately 25% of cases FVIII activities, ranging from 0.05 to even 0.40 kIU/L, can be detected.

Testing for the presence of FVIII autoantibodies (FVIII inhibitors) by using a standard Bethesda or the Nijmegen-modified Bethesda assay is the most important diagnostic procedure for the confirmation of AHA. In cases when determined FVIII activities are > 0.05 kIU/L, pre-heat inactivation of the patient's plasma at 56 °C for 30 minutes (to precipitate circulating residual FVIII) should be performed in order to improve the sensitivity of inhibitor detection. Residual FVIII activities after the incubation step, can be measured either using the one-stage clotting assay or the chromogenic method. The main characteristic of FVIII autoantibodies is that they display complex and non-linear type 2 kinetics, implicating that the assay may not be able to estimate the true potency of the autoantibody. By consensus, the dilution closest to a 50% inhibition of FVIII in normal plasma should be selected for the estimation of the inhibitor titer. Alternatively, FVIII autoantibodies (both neutralizing and non-neutralizing) could also be detected by using enzyme-linked immunosorbent assays. Finally, if recombinant porcine FVIII (rpFVIII) is a therapeutic option, then a Bethesda assay specific to rpFVIII should be considered as it may help guide treatment decisions.

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S1-4**Updated definition and scoring of disseminated intravascular coagulation in 2025**

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Disseminated intravascular coagulation (DIC) has been a common diagnosis made by health care givers since the dawn of the 20th century. The incidence of four common clinical scenarios traditionally associated with DIC, sepsis, trauma, obstetrical disorders, and cancers, are on the increase due to better diagnostics and management strategies. There is a varied understanding of the pathophysiology of this condition. In this perspective, the present reasons for this change in consideration and encourage caregivers to consider a DIC diagnosis at an early stage based on new criteria to help patients benefit from available treatments.

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S2 Modern approach to therapeutic drug monitoring

S2-1

Different faces of therapeutic drug monitoring

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The efficacy and toxicity of some drugs are difficult to assess based on clinical signs and symptoms alone. Numerous factors influence the pharmacokinetics of a drug and, consequently, its pharmacological response. An important tool in the management of pharmacotherapy is therapeutic drug monitoring (TDM), which involves the quantitative measurement of drug concentrations in biological samples. The main goals of TDM are to reduce pharmacokinetic variability, accelerate clinical improvement, and enhance drug tolerability and safety.

At the beginning of TDM application, only the concentrations of the parent drug were measured. In some cases, measuring drug concentrations alone is insufficient, and additional approaches may be required to optimize therapy and improve treatment outcomes. Drug biotransformation by enzymes can generate metabolites with pharmacodynamic properties that are either similar to or different from those of the parent compound. Some drugs are administered as prodrugs and exhibit little or no pharmacological activity until they are converted into active metabolites. For drugs and metabolites with similar pharmacodynamic effects, the sum of the concentrations of the parent compound and its active metabolite, referred to as the active moiety, is relevant for TDM guided dose adjustment. Measurement of inactive metabolites can also provide valuable information regarding enzyme phenotype during therapy. Furthermore, some metabolites have been shown to contribute to drug toxicity, making their monitoring important for assessing patient safety. For certain drugs, a clear relationship between dose, concentration, and therapeutic effect is not observed. In such cases, the area under the concentration-time curve (AUC) is considered a more appropriate pharmacokinetic parameter for TDM. However, routine determination of AUC in clinical practice is challenging because it generally requires multiple blood samples over time. To address this limitation, AUC can be estimated using a limited number of concentration-time points incorporated into a multiple linear regression equation.

Advances in analytical technologies and their implementation in clinical practice have enabled the measurement of an increasing number of drugs and metabolites that can be relevant for assessing pharmacokinetic phenotype which has become a valuable tool for individualized therapy. The classic approach to phenotyping is the application of probe drug, which were initially developed after the observation that drug metabolism is strongly influenced by genetic factors. Probe drugs are administered to assess the activity of specific drug metabolizing enzymes and thereby determine an individual's metabolic phenotype. Further progress in mass spectrometry based analytical methods has enabled the development of probe drug cocktails containing six or more compounds. These cocktails allow simultaneous quantification of the activity of multiple enzyme isoforms in a single test, providing a comprehensive assessment of drug-metabolizing capacity.

Despite the considerable potential of TDM and related pharmacokinetic monitoring approaches to optimize pharmacotherapy, their benefits can only be fully realized when these methods are appropriately integrated into the clinical decision-making process and routine patient care.

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S2-2

Clinical use of therapeutic drug monitoring for psychoactive drugs - from retrospective problem solving to clinical decision support

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Therapeutic drug monitoring (TDM) of psychoactive drugs has traditionally been used as a retrospective problem-solving tool, mainly to investigate nonresponse, adverse effects, suspected nonadherence, drug interactions, or altered pharmacokinetics. For many antidepressants, antipsychotics, mood stabilizers, and related agents, interindividual variability in absorption, metabolism, distribution, and elimination can lead to substantial differences in exposure despite standard dosing. Clinical effect and side effects of psychoactive drugs are not easily captured by objective measures, and mostly relies on self reporting from patients. As a result, plasma concentrations may provide clinically useful information that complements symptom assessment and guides individualized treatment.

In routine practice, TDM is most often applied after clinical concerns arise, when measured concentrations help explain treatment failure or toxicity. However, when integrated into structured dosing strategies and clinical decision support systems, TDM can support proactive treatment optimization. This is especially relevant for psychoactive drugs with narrow therapeutic ranges, active metabolites, pharmacogenetic influences, or strong exposure-response relationships. In such settings, concentration data can inform dose adjustment, and interpretation of apparent treatment resistance or intolerance.

A move from problem solving to clinical decision support would require standardized sampling and integrate interpretive algorithms that account for patient-specific factors such as age, comedication, smoking status, hepatic or renal impairment, and pharmacogenetic variation. Model-informed precision dosing may further enhance TDM by combining measured concentrations with pharmacokinetic models to estimate exposure and recommend individualized regimens. This approach may improve target attainment, reduce adverse drug reactions, and increase confidence in prescribing decisions.

Despite its promise, implementation of TDM for psychoactive drugs remains variable. Barriers include limited clinician familiarity, inconsistent access to assays, uncertainty regarding optimal timing of blood sampling, and incomplete evidence linking TDM-guided care to patient-centered outcomes. Greater integration with electronic health records and decision support tools may help address these challenges by making recommendations more accessible and actionable at the point of care.

In conclusion, TDM of psychoactive drugs is evolving from a reactive laboratory service into a more proactive tool for precision psychiatry. By supporting individualized dosing and timely clinical decisions, TDM has the potential to improve safety, efficacy, and treatment continuity in patients receiving psychoactive pharmacotherapy.

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S2-3

Clinical application of therapeutic monitoring of immunosuppressants – is there a place for improvement?

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Therapeutic drug monitoring (TDM) of immunosuppressive therapy is an essential component of post-transplant patient management due to the narrow therapeutic range of immunosuppressive agents, pronounced interindividual pharmacokinetic variability, and the significant risk of graft rejection or drug related toxicity associated with inadequate drug exposure. Calcineurin inhibitors, mammalian target of rapamycin inhibitors, and mycophenolic acid require regular monitoring to ensure optimal therapeutic efficacy while minimizing adverse effects. Although TDM is primarily established in solid organ transplantation, its role is increasingly being explored in selected autoimmune and chronic inflammatory diseases.

Recent clinical guidelines and expert consensus statements emphasize the importance of individualized immunosuppressive therapy based on drug exposure, patient specific pharmacokinetic characteristics, and long term graft outcomes. Although trough concentration monitoring remains the standard approach in routine clinical practice, increasing attention is being directed toward personalized strategies, including area under the concentration-time curve-guided dosing, minimization protocols, adherence monitoring, and pharmacogenetic data.

From an analytical perspective, liquid chromatography coupled with tandem mass spectrometry has become the reference method for immunosuppressant determination due to its superior analytical specificity, reduced susceptibility to metabolite interference, and the possibility of simultaneous analyte quantification. Compared with immunoassays, this approach provides improved analytical accuracy, particularly in complex clinical conditions and in patients receiving combination immunosuppressive therapy.

Implementation of unified analytical platforms capable of simultaneous determination of multiple immunosuppressants may further improve laboratory workflow, reduce turnaround time, and contribute to standardization of TDM.

Despite significant advances in both clinical and analytical aspects, several challenges remain. Preanalytical variables, including sampling time relative to drug administration and specimen handling, continue to influence result interpretation. Furthermore, differences between analytical methods and laboratories may affect result comparability and clinical interpretation of results, highlighting the ongoing need for harmonization and standardization.

Future developments are expected to focus on integrating pharmacokinetic, pharmacogenetic, and clinical data into personalized dosing strategies supported by advanced analytical technologies and multidisciplinary collaboration. Such an approach may further optimize immunosuppressive therapy, improve long-term transplant outcomes, and enhance the clinical value of TDM.

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S2-4

Complementary use of therapeutic drug monitoring (TDM) and pharmacogenomics - focus on drugs for which we lack TDM

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Interindividual variability in drug response remains a challenge in clinical pharmacotherapy, resulting in treatment failure, adverse drug reactions, or unnecessary healthcare costs. Personalisation of pharmacotherapy relies on two complementary tools: therapeutic drug monitoring (TDM) and pharmacogenomics (PGx). Therapeutic drug monitoring measures drug concentrations to guide dose adjustments in real-time, while PGx analyses genetic variants to predict drug response and adverse drug reactions (ADRs). While both strategies aim to optimise therapy, their relative contribution varies across therapeutic areas and drug classes.

Pharmacogenomics provides a genetic blueprint by analysing genetic variants in drug metabolising enzymes, transporters and other pharmacogenes such as *ABCG2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *HLA-A*, *HLA-B*, *DPYD*, *UGT1A1*, *NUDT15*, *TPMT*, *VKORC1*, *SLCO1B1* and others, to predict individual responses and guide drug and dose selection before treatment begins, or to retrospectively resolve problems in pharmacotherapy. Therapeutic drug monitoring measures drug concentrations in blood and biological fluids to guide real-time dose adjustments by capturing dynamic factors that genetics cannot account for, including drug-drug interactions, patient adherence, organ function, inflammation, and physiological changes. Traditionally, TDM has been considered the gold standard for drugs with a narrow therapeutic index and established concentration–response relationships, such as tacrolimus, selected antiepileptic drugs and antipsychotics. However, for many commonly prescribed medications, including antidepressants, antipsychotics, fluoropyrimidines, anticancer agents, analgesics, and others, routine TDM is either unavailable, insufficiently standardised, or not implemented in clinical practice. In these settings, pharmacogenomic testing may provide valuable information before treatment initiation and serve as an alternative or complementary tool for dose individualisation. This lecture will review the scientific basis and clinical applications of combined TDM-PGx approaches, with a focus on therapeutic areas where TDM remains limited or unavailable. Practical examples from psychiatry, oncology, infectious diseases, and transplantation medicine will be presented. The integration of pharmacogenomic markers and drug concentration measurements represents a promising way toward truly personalised pharmacotherapy and improved patient outcomes.

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S3 Allergic diseases – pandemics of the future

S3-1

Laboratory diagnostics of allergies using modern technologies

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Allergic diseases are chronic conditions with increasing prevalence, morbidity, and impact on quality of life, representing a significant public health concern and economic burden. Accurate diagnosis is essential for effective management. Diagnosing allergic diseases requires a comprehensive approach, utilising both *in vitro* and *in vivo* diagnostic tests. Laboratory assessment primarily focuses on detecting specific immunoglobulin E (sIgE) antibodies to allergens that, when interpreted alongside a detailed patient medical history, are suspected to trigger clinical symptoms. Historically, laboratory allergy diagnostics have relied on identifying sensitisation to specific allergens, *i.e.*, identifying the presence of sIgE to native allergen extracts. These extracts are inherently heterogeneous and complex, containing a mixture of proteins and non-protein components, including polysaccharides and lipids, that can cross-react with proteins from unrelated sources. In the past decade, the adoption of component-resolved diagnostics (CRD) in clinical practice has significantly improved the precision of sensitisation profiling. Component-resolved diagnostics enables the identification of clinically relevant allergen components associated with true allergy and supports risk assessment for severe anaphylactic reactions. Component-resolved diagnostics provides several advantages compared to native allergen extracts:

1. Increased diagnostic sensitivity for symptoms caused by allergenic molecules present in low quantities or with reduced stability, which may be missing from traditional extracts
2. Enhanced diagnostic specificity due to the reduced complexity of individual allergen components
3. Ability to distinguish primary sensitisation from cross-reactivity
4. Improved identification of cross-reactivity between allergens
5. Useful for risk assessment in food and insect venom allergies, including predictive value for allergy development in pediatric patients.

Recently, multiplex-specific immunoglobulin E (IgE) testing technologies have emerged, advancing the field of laboratory allergy diagnostics. These platforms integrate modern biotechnological and digital innovations, allowing a transition from extract-based testing to molecular-level and functional allergen profiling. This shift enables more comprehensive patient evaluation and management. Multiplex chip-based assay technologies, such as ImmunoCAP ISAC and ALEX2/3, can simultaneously detect sIgE to hundreds of individual allergen components using a minimal volume of serum.

As multiplex component-resolved diagnostics become increasingly available worldwide, and as innovative approaches evolve, CRD, combined with advanced technologies, will become integral to personalised medicine. These advancements are expected to optimise diagnostic workflows, enhance patient safety, and improve the efficacy of targeted allergy therapies.

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S3-2

Exploring the latest innovations in laboratory diagnostics of anaphylaxis: towards personalized approaches and biomarker development

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Anaphylaxis is a rapidly progressing and potentially life-threatening systemic hypersensitivity reaction that may involve respiratory, cardiovascular, cutaneous, and gastrointestinal manifestations. Common triggers include foods, insect venom, and medications, particularly antibiotics and neuromuscular blocking agents used during anesthesia. Despite advances in clinical recognition and management, laboratory confirmation of anaphylaxis remains challenging because of the heterogeneity of clinical presentations and the limitations of currently available biomarkers.

Serum tryptase remains the principal biomarker for confirming mast cell activation during anaphylaxis. Acute tryptase levels are typically measured within 15-120 minutes after symptom onset and compared with baseline levels using the formula "baseline tryptase + 20% + 2 µg/L". Elevated baseline tryptase levels, hereditary alpha-tryptasemia, and clonal mast cell disorders have been associated with increased risk and severity of anaphylactic reactions. However, serum tryptase has important limitations, as elevated concentrations may be absent in up to 30% of reactions, particularly in food-induced and drug-induced anaphylaxis. Consequently, there is an increasing need for novel biomarkers with improved sensitivity and specificity.

Among emerging biomarkers, the chemokine CCL2 has shown considerable promise. CCL2 is involved in basophil chemotaxis and migration from peripheral blood into target tissues during allergic reactions. Studies have demonstrated that serum CCL2 concentrations may correlate with both the presence and severity of anaphylaxis. Comparative analyses revealed that serum tryptase demonstrates higher overall sensitivity and specificity than CCL2 alone; however, the combined sequential assessment of both biomarkers significantly improves diagnostic accuracy. Combined biomarker testing demonstrated particularly high sensitivity in food- and insect-venom-induced anaphylaxis, supporting the value of multimodal biomarker evaluation.

Functional cellular assays have also become increasingly important in allergy diagnostics. The basophil activation test (BAT) evaluates the biological activity of allergen-specific IgE through FcεRI-mediated basophil activation and degranulation. Compared with serum-specific IgE testing, BAT provides functional evidence of clinically relevant sensitization. The basophil activation test demonstrates high specificity, especially in perioperative hypersensitivity reactions involving neuromuscular blocking agents, antibiotics, chlorhexidine, and opioids. Nevertheless, its limited sensitivity means that negative results cannot reliably exclude future reactions.

In addition to BAT, mast cell activation testing (MAT) has emerged as a promising research tool for predicting reaction severity and enabling personalized diagnostic approaches. Perioperative hypersensitivity reactions remain a major clinical challenge because of their unpredictable nature and the frequent involvement of multiple potential allergens.

Comprehensive diagnostic evaluation, therefore, includes clinical history, anesthesia records, skin testing, serum tryptase, specific IgE determination, BAT, and selected drug provocation tests.

Recent advances in biomarker discovery and functional cellular assays are significantly improving the laboratory diagnosis of anaphylaxis. Future diagnostic strategies will likely combine clinical phenotyping with sequential biomarker assessment and cellular activation testing to support personalized diagnostics, improved risk stratification, and safer patient management.

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S3-3

The role of laboratory diagnostics in identification, monitoring and treatment of patients with allergies

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A wide variety of allergenic proteins can lead to allergic sensitization. These proteins are called allergens and originate from various allergen sources. For example, Bet v 1, Bet v 2, *etc.*, are different allergens (or allergen components) found in birch pollen (the allergen source).

Some allergens from different sources can be classified in the same allergen group, due to their structural similarity. These are called homologous allergens, and are responsible for cross-reactivity. That means that sIgE antibodies induced by one allergen (*e.g.* Bet v 1 from birch pollen) can bind to another, structurally similar, allergen (*e.g.* Mal d 1 from an apple), and thus cause cross-reactions (*e.g.* oral allergy syndrome in a patient allergic to birch pollen, when consuming an apple).

In some instances, it is important to determine the patient's sensitization profile, *i.e.* which components of the allergen source led to the sensitization. This so-called component-resolved diagnosis (CRD) helps differentiate between double sensitization and monosensitization with cross-reactivity. For example, a patient with symptoms of allergic rhinitis during spring, with positive skin prick test (SPT) and sIgE results for both birch and grass pollens, represents a frequent diagnostic challenge in clinical allergy practice. Why? Because there are three possibilities - double sensitization to birch and grass pollens (positive sIgE against Bet v 1, positive sIgE against Phl p 1 and/or Phl p 5), monosensitization to birch pollen with cross-reactivity (positive sIgE against Bet v 1, positive sIgE against profilin and/or polcalcin, but negative sIgE against Phl p 1 and/or Phl p 5), and monosensitization to grass pollen with cross-reactivity (positive sIgE against Phl p 1 and/or Phl p 5, positive sIgE against profilin and/or polcalcin, but negative sIgE against Bet v 1). And why is this distinction important? In this example, knowing the patient's sensitization profile is crucial for selection of appropriate allergen extract(s) for allergen immunotherapy (AIT), *i.e.* whether a patient should be treated with a birch- or a grass-pollen extract or both. In mold-sensitized patients, CRD can also help differentiate between those with simple fungal colonization (positive sIgE against Asp f 1 and/or Asp f 3) and those with clinically relevant *Aspergillus*-related disease, *e.g.* allergic bronchopulmonary aspergillosis (ABPA), (positive sIgE against Asp f 2 and/or Asp f 4 and/or Asp f 6), which, again, influences therapeutic decisions.

Another important role of laboratory diagnostics is in monitoring patients receiving AIT. The efficacy of therapy correlates strongly with an increase in sIgG or sIgG4 antibody titers. Therefore, close collaboration between laboratorians and clinicians is essential for accurate diagnosis, selection of therapy, and monitoring of treatment response in allergic patients.

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S3-4

New diagnostic biomarkers in allergology: the path towards personalized allergy diagnostics

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Allergic diseases constitute a significant medical and socio-economic burden, as evidenced by their increasing global prevalence. These conditions often progress in a sequential pattern known as the "allergic march", beginning with atopic dermatitis, advancing to food allergies, and culminating in allergic rhinitis and childhood asthma. The incidence of allergic diseases has risen steadily, now impacting approximately 20% of the global population, particularly among children, and is considered both an epidemic and a pandemic concern. This escalation is attributed to a complex interplay of environmental factors, including climate change and air pollution, as well as immune system dysregulation related to dietary modifications, alterations in the microbiome, and broader lifestyle changes associated with the "hygiene hypothesis".

Diagnosing allergic diseases is challenging due to the complex mechanisms underlying allergic reactions and the broad spectrum of symptoms. Traditional diagnostic algorithms rely on comprehensive medical examinations, combined with laboratory diagnostics. Currently, the primary diagnostic tests involve measuring total immunoglobulin E (IgE) and specific immunoglobulin E (sIgE), which quantify the immune system's response to allergens. Due to limited diagnostic specificity and cross-reactivity, traditional diagnostics are being transformed and are now routinely supplemented by analysis of the molecular components of allergens (cIgE).

Component-resolved diagnostics (CRD) utilizes recombinant or highly purified native allergen molecules to identify the precise protein driving the allergic response. This distinguishes genuine sensitization from harmless cross-reactivity and gives valuable information for precise therapy. Considering CRD and the strengths and limitations of extract-based diagnostics, the literature proposed introducing mathematical ratios sIgE/IgE and cIgE/sIgE into clinical practice. These relations provide information on the share of sensitization attributable to the whole extract or show how much a specific allergenic component contributes to sensitization to the whole extract, suggesting sensitization to an untested/unknown component.

In addition to expanded diagnostics based on IgE antibodies in singleplex or multiplex formats, new laboratory markers include advanced cellular tests of basophil/mast cell activation, as well as new inflammatory proteins such as periostin. Additionally, future application of epigenetics in personalized allergology is also notable. MicroRNAs (miRNAs) are critical regulators of numerous biological processes, including cell proliferation, differentiation, and apoptosis. Recent research has increasingly explored miRNA expression as a potential biomarker for the diagnosis, treatment, and prognosis of prevalent chronic diseases, including allergies. For example, individuals with asthma exhibit elevated serum expression of several miRNAs, which are implicated in modulating proinflammatory cellular functions. However, current studies are still limited by small sample sizes, heterogeneous study populations, and varied methodologies, highlighting the necessity for diagnostic accuracy studies to establish definitive correlations among miRNA profiles and clinical outcomes.

The diagnostic approach nowadays is evolving toward molecular allergology, epigenetic profiling, and cellular response markers, making the path towards personalized allergy diagnostics. Future laboratory diagnostics will not rely on a single biomarker – but will integrate patients' genetic predispositions and environmental triggers with skin prick tests, IgE concentrations, CRD, miRNA profiles and other relevant findings to identify hidden correlations and predict allergic responses and therapy options more reliably than traditional diagnostic methods.

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S4 Women in sports – a multidisciplinary challenge

S4-1

Can a single hormone define sex: fair play or illusion in sport?

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Sex determination in sport included invasive physical examinations performed in 60-es which were replaced by 30 years of genetic testing (mostly Barr body test and later PCR detection of *SRY* gene). Still, misclassification, especially in androgen insensitivity syndrome and sex development disorders, lead to increase of criticism. In late nineties the genetic era stopped, when the International Olympic Committee eventually dismissed obligatory gene sex testing around Olympics in Sydney in year 2000. In 21st century, hormonal biomarkers, especially testosterone, came in focus as the endocrine marker with strongest anabolic and erythropoietic functions. For a moment eliminating unfair advantage of men seemed promising, as these two effects are dominantly responsible for anaerobic (strength and power) type of sports or disciplines and also aerobic (endurance) events. However, can the concentration of one hormone, testosterone, really define someone's sex? Current rules in elite sport, including associations like World Athletics, World Aquatics, L'Union Cycliste Internationale now use/or have used a compromise value, a threshold of around 5 nmol/L serum testosterone to determine the eligibility for participating in female events. Whether this approach is ethically and scientifically fair and valid is largely questioned. The ratio behind was that upper range of female testosterone concentrations are usually ~0.5–2 nmol/L and still below male range (~8–30 nmol/L). From a physiological standpoint, testosterone increases muscle mass and power, red blood cell concentration, and recovery, all of which are necessary for performance. The significant differences in average circulating concentrations between males and females, seemed to justify its use in classification but testosterone and performance have a non-uniform relationship.

Variability in androgen sensitivity, 5 α -reductase as a signal and sex hormone binding globulin influence bioavailability. Athletes with differences of sexual development may still have high testosterone with no receptor sensitivity, and no advantage. This also creates injustice for female with naturally high testosterone, as it can exclude individuals who have advantage by their biological variation. Finally, puberty in males involves high rise of testosterone with permanent structural changes in tissues, especially in bone, muscle mass, bone geometry, increased vital capacity, larger stroke and cardiac output. Lowering of someone's testosterone would not erase the inevitable advantage in most of the disciplines and would not reverse musculoskeletal and cardiorespiratory gains during puberty. From biochemical standpoint, differences in the methods of hormone assays and external effects, like training, stress, and circadian rhythms, change measured testosterone concentrations. So, the question of reliability should be raised. Also, from ethical and medical point, reliance on a single hormone leads to interventions of lowering testosterone to meet eligibility criteria which might have cardiovascular, metabolic, and psychological consequences.

While testosterone is in use in the classification of athletes, it cannot singularly define sex in a physiologically sensible and logical way. The 5 nmol/L threshold is administrative invention and should be understood only as a regulatory measure failing to provide definitive biological cut-off. Integrating broader anamnestic, biochemical, genetic, and physiological data to better reflect the complexity of human sex and strongly focus to fairness in competition should be considered in future.

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S4-2

Female athlete $\neq$ male athlete: the impact of the menstrual cycle on sports performance

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Combat sports such as wrestling and judo have traditionally been developed within a male sports system, in which training models, loading methods and competition structures have been primarily shaped according to the physiological and sociological characteristics of men. The inclusion of women in these sports has highlighted the need to critically re-examine approaches to training female athletes, particularly in the context of hormonal changes associated with the menstrual cycle. The aim of the lecture is to present the specific characteristics of training female wrestlers and judokas, with emphasis on the influence of the menstrual cycle on sports performance, subjective readiness and psychophysical functioning in an environment that largely lacks female coaches and sex specific training routines.

The menstrual cycle is a complex physiological process characterized by cyclic changes in estrogen and progesterone concentrations that can affect mood, perception of fatigue, pain threshold, thermoregulation, sleep quality and the perceived readiness for training and competition. Although research findings on the direct effect of the menstrual cycle on objective indicators of sports performance are not entirely consistent, systematic reviews and experimental studies suggest a possible small reduction in performance in the early follicular phase, with pronounced individual variability. At the same time, a large proportion of female athletes subjectively report decreased readiness and increased symptoms during the premenstrual days and at the onset of menstruation. In high intensity, full contact sports such as wrestling and judo, these changes may have a substantial practical impact on training quality, tactical decision making, pain tolerance and emotional stability during combat.

An additional problem is that in many wrestling and judo environments, training programmes for female athletes are still based on models developed for male populations, without systematic monitoring of the menstrual cycle and without structured communication about menstruation related symptoms. The lack of female coaches further hinders open discussion of these issues, so athletes often hesitate to report menstrual problems to male coaches. Such an environment can lead to the concealment of symptoms, the absence of timely adjustment of training loads and increased psychological strain, especially during periods of intensive training, body mass reduction or key competitions.

This lecture highlights the need for an individualized approach to training female athletes through menstrual cycle tracking, flexible planning of training loads and systematic coach education on the specificities of women's sport. The goal of such an approach is not to lower training demands, but to optimize performance and preserve athletes' health in weight category and high contact sports. Systematic symptom monitoring and open communication can enable timely adjustments of training volume and intensity, more effective planning of weight reduction processes and better understanding of individual responses to load. In contemporary sport, it is essential to abandon the notion that women are "smaller versions of men" and to develop training models that integrate the biological, psychological and sociocultural specificities of female athletes in combat sports.

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S4-3

HEMSTER study: the role of medical biochemist in sports medicine

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Laboratory medicine plays an increasingly important role in sports medicine, not only for detecting disease, but also for monitoring physiological adaptation to exercise and training. However, interpretation of laboratory results in athletes remains challenging because standard reference intervals are usually derived from the general population and may not reflect the unique physiology of trained individuals. Intensive physical activity causes significant functional and metabolic changes and adaptations in the athlete's organism. These adaptation changes encompass cells, tissues, organs, as well as total body physiological changes. The observed physiological changes result in scarcely described altered biochemical and hematological parameters. There is a significant under-representation of women in sport and exercise medicine research. Our intention, through a series of studies under the HemSter Study, was to address women in sports and their unique differences to fill this gap. The HemSter Study was the first to win the Croatian Society of Medical Biochemistry and Laboratory Medicine (CSMBLM) program to encourage scientific and research activities of CSMBLM members, „Prof. Dr. Elizabeta Topić“. The award enabled this very important research, under which important findings were published in the respected scientific journals. Most findings were concentrated upon the applicability of the existing harmonized reference intervals to this specific population of young female athletes. One important example is testosterone concentration in female athletes. Testosterone contributes to muscle hypertrophy, strength development, and exercise adaptation. In the HemSter study, testosterone concentrations were evaluated in 121 healthy female athletes from different team sports using liquid chromatography-tandem mass spectrometry and six commercial immunoassays. The study demonstrated that several manufacturer-provided reference intervals did not apply to female athletes because a substantial proportion of athletes had testosterone values above the conventional upper reference limit despite being healthy. Similar findings were found for hematological and coagulation parameters, where C-reactive protein and fibrinogen differed significantly from the available common national reference intervals for the healthy non-athletes' population. Additionally, a higher cut-off for D-dimers was found in a group of healthy young female athletes, although this should be extensively verified before implementation for venous thromboembolism diagnosis exclusion. Another very important question that the HemSter study raised was that biological variability further complicates the interpretation of laboratory markers in athletes. Exercise-induced changes in creatine kinase, liver enzymes, and inflammatory markers may reflect physiological muscle remodeling rather than tissue injury or disease. Creatine kinase is particularly sensitive to exercise intensity, muscle mass, training status, and timing of blood sampling. Similarly, aminotransferases such as aspartate aminotransferase and alanine transaminase may increase after strenuous exercise because of skeletal muscle release, potentially mimicking liver pathology. Therefore, isolated abnormal laboratory values in athletes should always be interpreted within the context of recent training load, recovery status, and sport discipline. In conclusion, athletes represent a physiologically distinct population whose laboratory findings frequently differ from those of the general population. Conventional reference intervals may therefore lead to false-positive interpretations and unnecessary diagnostic procedures. Athlete-specific reference intervals, longitudinal monitoring, and awareness of biological variability are essential for accurate interpretation of biochemical and hormonal markers in sports medicine.

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S4-4

Beyond “bikini” fitness: effective exercise programming for women’s health

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While the efficacy of diverse exercise modalities, ranging from high-intensity interval training (HIIT) to resistance conditioning, in improving cardiometabolic markers and hormonal balance is well-documented, a significant gap remains between clinical potential and real-world implementation. Large-scale meta-analyses involving millions of participants demonstrate that global adherence to combined aerobic and muscle-strengthening guidelines remains suboptimal. This discrepancy is particularly pronounced in female populations, where exercise programming is frequently overshadowed by the “bikini” fitness paradigm. This aesthetic-centric model prioritizes extrinsic outcomes, such as weight loss and specific body compositions, over holistic physiological health and longevity.

The primary challenge in female-focused physical activity interventions is the high attrition rate driven by these extrinsic motivators. Evidence suggests that when the primary motive for exercise is exclusively appearance-based, long-term commitment is markedly lower. This approach often leads to psychological burnout and a cyclic pattern of exercise cessation, which undermines the preventative health benefits and cardiovascular protection afforded by regular physical activity. Furthermore, extraordinary claims regarding the adherence benefits of time-efficient modalities like HIIT have been scrutinized, suggesting that high-intensity protocols may not necessarily foster better long-term engagement compared to more sustainable approaches.

To ensure that women remain engaged in physical activity throughout their lifespan, a shift toward a health-centric, functional programming model is essential. It is often difficult for women to find dedicated time for exercise due to competing professional and personal demands. However, research emphasizes that lifelong endurance exercise is vital for maintaining a youthful cardiovascular system and high aerobic capacity as women age. To improve adherence, exercise programs must be designed to be time-efficient and easy to master, moving the focus away from “exercising for appearance” toward “training for functional longevity”. By rebranding physical activity as a tool for metabolic stability and resilience, women can better sustain a movement culture that lasts throughout their lives. This shift ensures that the extra years women live are characterized by high health status and independence rather than frailty.

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S5 MicroRNA in germ cell tumors– joint symposium with Horizon Project Attract Adria

S5-1

Germ cell tumors

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Testicular tumors account for approximately 5% of urological tumors and affect younger men. They are predominantly testicular germ cell tumors (TGCTs), which are histologically classified into seminomas and non-seminomatous germ cell tumors (NSGCTs). Although histological classification remains the basis for diagnosis and therapeutic decision-making, serum markers such as lactate dehydrogenase (LD), alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin (β -hCG) are used in clinical assessment. However, their sensitivity is limited, particularly in localized disease and in seminoma. For this reason, increasing attention has been directed in recent years toward circulating microRNAs.

MicroRNA-371a-3p is a small non-coding RNA associated with embryonic pluripotency and the biology of germ cell tumors. In the literature, it is described as a highly specific biomarker of TGCTs, with better diagnostic sensitivity and specificity than conventional serum markers. Its most important feature is the detection of active and undifferentiated tumor mass.

Therefore, serum miR-371a-3p levels generally correlate better with tumor volume, clinical stage and the presence of viable disease than with subtle histological differences.

Nevertheless, the association between miR-371a-3p and TGCT histology is biologically important. It is strongly expressed in seminomas and in most non-teratomatous components of NSGCTs, including embryonal carcinoma, yolk sac tumor and choriocarcinoma. In some analyses, seminomas show higher tissue expression than non-seminomatous tumors, with differences also observed among NSGCT subtypes. Teratoma shows very weak or absent miR-371a-3p expression, which represents the well-known “teratoma gap”. Although expression can be detected in GCNIS, serum detection of an isolated intraepithelial lesion is not sufficiently reliable for clinical diagnosis. In addition, in very small seminomas, particularly those smaller than 1 cm, the result may be negative despite the presence of tumor.

Our experience to date with the introduction of miR-371a-3p analysis in patients with testicular tumors treated at Sestre milosrdnice University Hospital Centre is consistent with published data. At initial presentation, miR-371a-3p appears to be more sensitive than conventional markers, particularly in patients with normal LDH, AFP and β -hCG values. Its levels correlate better with tumor volume and lesion size than with the distinction between seminoma and NSGCT. After orchiectomy, a decrease or normalization of miR-371a-3p values is expected in localized disease, whereas a persistently positive result may indicate residual, occult or metastatic viable disease. Such a finding is particularly clinically relevant in stage I patients with pathological risk factors for relapse, such as rete testis invasion or larger tumor size in seminoma, and lymphovascular invasion in NSGCT. Nevertheless, miR-371a-3p should currently be interpreted as an additional biomarker alongside histology, stage, radiological assessment and conventional serum markers, rather than as an independent criterion for changing therapeutic decisions.

In conclusion, miR-371a-3p represents the most promising novel serum biomarker for TGCTs. Its greatest value lies in the detection of viable non-teratoma tumor components, assessment of tumor burden, monitoring of treatment response and potential earlier identification of relapse. The main limitations of the method are poor detection of teratoma and isolated GCNIS, as well as the possibility of false-negative results in very small tumor lesions.

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S5-2

MicroRNA-371 in testicular cancer – a laboratory perspective

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Testicular germ cell tumours (TGCTs) are the most common malignancies in young adult men. Well established serum tumour markers such as alpha-fetoprotein (AFP), beta-human chorionic gonadotropin (β -hCG), and lactate dehydrogenase (LD) remain part of routine clinical practice but their limited sensitivity and specificity to detect a substantial proportion of active tumours have created a need for more reliable biomarkers.

MicroRNA-371a-3p (miR-371a-3p, miRNA-371) has emerged as the most promising molecular biomarker for TGCTs. This embryonic stem cell-associated microRNA is highly expressed in most seminomatous and non-seminomatous germ cell tumours and can be detected in peripheral blood. Unlike conventional biomarkers, miRNA-371 reflects tumour-specific gene expression and offers a direct molecular approach to disease detection and monitoring.

MicroRNA-371 measurement represents a shift toward routine molecular diagnostics in TGCTs. This biomarker is quantified in serum using reverse transcription quantitative PCR (RT-qPCR), following extraction of circulating microRNA. Analytical workflows incorporate endogenous reference microRNAs, negative controls, and relative quantification approaches, ensuring robust and reproducible measurements. The assay requires small serum volume and demonstrates excellent analytical sensitivity, linearity, and inter-laboratory comparability. Preanalytical quality is particularly important because circulating microRNAs are susceptible to interference from hemolysis and improper sample handling. Standardised protocols for blood collection, serum preparation, storage, RNA extraction, and quality control are therefore essential for reliable implementation in routine laboratory practice.

Clinically, miRNA-371 significantly outperforms conventional serum tumour markers. Results of large multi-centre study have shown a sensitivity of 91.8% and a specificity of 96.1% for the detection of active TGCTs. High diagnostic accuracy was observed in both seminoma and non-seminoma. During surveillance, miRNA-371 has shown superior sensitivity for recurrence detection compared with conventional serum markers. In a recent prospective study, all clinically confirmed relapses were detected by miRNA-371, whereas AFP and β -hCG identified less than half of recurrence cases.

Although miRNA-371 demonstrates excellent diagnostic performance in TGCTs, pure teratomas generally lack miR-371a-3p expression and therefore cannot be detected using this biomarker. Moreover, miRNA-371 should be used as an adjunctive rather than a stand-alone biomarker and its results should always be interpreted within the broader clinical context.

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S5-3

Results of miRNA-371 in patients with testicular cancer

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Testicular germ cell tumors are the most common solid malignancy in young men. Although cure rates are high, accurate assessment of disease activity remains essential for diagnosis, treatment planning and surveillance. Conventional serum markers, including alpha-fetoprotein (AFP), beta-human chorionic gonadotropin (β -hCG) and lactate dehydrogenase (LD), have limited sensitivity and specificity. Circulating microRNA-371a-3p has emerged as a promising biomarker for viable germ cell tumors, with potential advantages in initial evaluation and post-treatment monitoring, although its performance is limited in teratoma.

We conducted a single-center observational analysis of patients treated for testicular tumors at the Department of Urology, University Hospital Centre Sestre milosrdnice. Serum LD, AFP, β -hCG and miRNA-371a-3p were assessed at initial presentation and after inguinal orchiectomy. Marker dynamics were evaluated in relation to histopathology, clinical stage and postoperative course during follow-up.

Preliminary experience indicates that preoperative miRNA-371a-3p was more frequently elevated than conventional serum markers. Following orchiectomy, miRNA-371a-3p generally decreased in patients with localized disease, while persistent or recurrent elevation prompted correlation with stage, histopathological findings and the need for additional diagnostic or therapeutic procedures. Conventional markers were elevated only in a subset of patients, supporting their limited sensitivity in routine practice. No consistent association between miRNA-371a-3p levels and a specific histological subtype was observed, and interpretation remained cautious in tumors containing teratoma.

Our initial clinical experience supports miRNA-371a-3p as a useful biomarker in the management of testicular germ cell tumors. Its incorporation into clinical algorithms may improve diagnostic confidence and follow-up assessment, but further analysis, methodological standardization and longer follow-up are required to define its role in specific clinical scenarios.

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S5-4

The role and value of miRNA-371 in patients with germ cell tumors – an oncologist's perspective

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Testicular germ cell tumors (GCTs) represent the most common malignancy among young males aged 20-40 years. Over recent decades, therapeutic management of GCTs has significantly improved, leading to cure rates exceeding 95% in patients with newly diagnosed disease. Most patients achieve long-term survival with a high quality of life and near-normal life expectancy. Nevertheless, important challenges remain, including the reduction of long-term treatment-related toxicity, minimizing patient burden, and improving the organization and accessibility of cancer care.

Germ cell tumors are considered a paradigm for the use of serum tumor markers in clinical oncology. However, conventional markers such as alpha-fetoprotein (AFP), beta-human chorionic gonadotropin (hCG), and lactate dehydrogenase (LD) exhibit limited sensitivity and specificity, reducing their overall diagnostic and prognostic utility. In the past decade, microRNA-371a-3p (miR371) has emerged as a promising novel biomarker. With a sensitivity of approximately 90% and a specificity exceeding 90%, miR371 significantly outperforms traditional serum tumor markers in testicular germ cell tumors.

MicroRNAs (miRNAs) are small noncoding RNA molecules, approximately 22 nucleotides in length, that regulate gene expression at the post-transcriptional level and participate in RNA silencing. They play a key role in epigenetic regulation of protein synthesis and may also contribute to carcinogenesis, functioning as oncogenes or tumor suppressors. It has been demonstrated that active germ cell malignancies release miR371 into the bloodstream, where it can be reliably detected in serum and plasma.

Retrospective studies suggest that miR371 exhibits several characteristics of an ideal biomarker. Its expression is detected in nearly all patients with intact primary GCTs and rapidly declines after surgical removal of the tumor in patients with clinical stage I disease. In contrast, miR371 levels persist or increase in patients with untreated metastatic disease. This dynamic profile makes miR371 particularly valuable for monitoring disease activity and treatment response.

The clinical applications of miR371 are broad and potentially transformative. It may facilitate the preoperative differentiation between benign and malignant testicular masses, potentially reducing the need for intraoperative frozen section analysis or even surgical exploration in selected cases. Additionally, it can aid in the evaluation of equivocal lymphadenopathies, thereby limiting the need for invasive diagnostic procedures and costly imaging techniques. During follow-up, miR371 measurement may enable earlier detection of relapse and reduce the frequency of imaging studies, decreasing both patient burden and healthcare costs.

Furthermore, due to its short half-life, miR371 allows for rapid assessment of chemotherapy response, identifying non-responding patients early and supporting timely modification of treatment strategies. It may also assist in decision-making regarding post-chemotherapy residual masses, potentially reducing the need for additional surgical interventions in selected patients, although limitations related to teratoma detection must be considered.

In conclusion, the discovery of circulating miRNAs from the miR371-3 cluster represents a major breakthrough in the field of testicular germ cell tumors. Ongoing research is expected to further validate its clinical utility and support its incorporation into future international guidelines, with the potential to improve diagnostic accuracy, optimize patient monitoring, and enable more personalized and less invasive management strategies.

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Working groups symposiums

WG-1 Symposium of the Working group for laboratory hematology

Platelets: small players, big challenges

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Platelets remain among the most preanalytically vulnerable parameters in laboratory medicine, with pseudothrombocytopenia, platelet clumping, and sample-related variability representing persistent diagnostic challenges. This symposium, convened by the Working Group for Laboratory Hematology of the Croatian Society of Medical Biochemistry and Laboratory Medicine (WG), focuses on harmonization of laboratory practice, presenting national recommendations for suspected EDTA-induced pseudothrombocytopenia and providing practical strategies to improve the reliability of platelet counting.

Given the large heterogeneity of practices and the aim of harmonizing the management of samples with suspected EDTA-induced pseudothrombocytopenia (PSTP), national recommendations were issued in collaboration with the Croatian Chamber of Medical Biochemists. A comprehensive protocol with detailed descriptions of all the activities that should be undertaken is presented, from defined criteria for raising suspicion on PSTP, exclusion of preanalytical errors that can affect platelet count, microscopic confirmation of PSTP, use of alternative samples (*i.e.* citrated venous blood sample) for obtaining platelet count, and uniform reporting.

A common question in routine practice remains whether a centrifuged citrated venous blood sample, already used for routine coagulation analysis, can be reliably used to verify the platelet count in thrombocytopenic patients. Our research has shown no statistically significant difference in the number of leukocytes, erythrocytes, hemoglobin, platelets, or mean platelet volume between blood samples with EDTA and sodium citrate anticoagulant, whether analyzed before or after centrifugation.

Besides EDTA-induced PSTP, platelet clumping is commonly caused by preanalytical factors, such as traumatic venipuncture, slow blood flow, or inadequate sample mixing. Many studies have evaluated vortexing as a potential method for resolving platelet clumping in EDTA samples, thereby reducing the need for repeat blood sampling. Our study confirmed previous findings that vortexing completely resolves platelet clumping in a significant proportion of samples.

The results of this work support the potential inclusion of vortexing in future national recommendations as part of the protocol for the management of such samples.

Another important challenge in platelet counting is the differences between capillary and venous blood samples. Capillary blood is more susceptible to preanalytical variability, including hemoconcentration, platelet aggregation, tissue fluid contamination, and local cell activation during skin puncture. Consequently, capillary samples may show either higher or falsely lower platelet counts compared to venous blood due to platelet aggregation and microclot formation. Despite these known differences, venous reference intervals are commonly applied to capillary samples in routine practice. This issue is particularly important in oncology, hepatology and pediatric patients, in whom therapeutic decisions frequently depend on accurate platelet counts. Inaccurate capillary results may lead to unnecessary treatment delay or inappropriate clinical management. One of the many WG goals was also to explore the possible differences in platelet count between capillary and venous samples by employing different platelet count methodologies.

The work of the national WG has been pivotal in harmonizing approaches to platelet testing and reducing preanalytical errors in routine practice. Through evidence-based recommendations, collaborative research, and standardized protocols for pseudothrombocytopenia and platelet clumping, the WG significantly contributes to improved diagnostic reliability, patient safety, and consistent laboratory practice nationwide in this field.

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WG-2 Symposium of the Working group for laboratory endocrinology**National recommendations of the Croatian Society of Medical Biochemistry and Laboratory Medicine: Thyroid function tests from the laboratory point of view**

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Thyroid function tests (TFTs) are important diagnostic tools for evaluating thyroid gland disorders and monitoring thyroid cancer. These tests include thyroid-stimulating hormone (TSH), total and free triiodothyronine (T3, FT3), total and free thyroxine (T4, FT4), thyroglobulin (Tg), and thyroid antibodies such as thyroid peroxidase antibodies (anti-TPO), thyroglobulin antibodies (anti-Tg), and thyroid-stimulating hormone receptor antibodies (anti-TSHR). A national survey conducted in Croatia in 2022 showed significant variability in laboratory practices, and the Croatian Society of Medical Biochemistry and Laboratory Medicine developed standardised recommendations covering the preanalytical, analytical, and postanalytical phases of testing.

In the preanalytical phase, proper patient preparation and sample collection are required to ensure reliable results. Blood samples should ideally be collected from fasting patients, since food intake may reduce TSH concentrations and potentially lead to misinterpretation of thyroid status. Patients are also advised to avoid strenuous physical activity for 24 hours before testing because exercise can temporarily alter thyroid hormone concentrations. Due to the circadian rhythm of TSH secretion, blood collection is recommended between 7:00 AM and 11:00 AM. Additionally, patients receiving thyroid hormone therapy should have their blood drawn before taking medication to prevent transient increases in hormone concentrations that could affect results. Laboratories should also document exposure to iodinated contrast agents or other procedures affecting thyroid function within the previous 1–3 months. Proper storage and stability conditions for samples must also be defined by each laboratory.

In the analytical phase, laboratories must verify the measurement methods before implementing them in routine practice. Verification procedures include evaluating precision, method comparison, and measurement uncertainty. Internal quality control materials should cover clinically important ranges, particularly low concentrations of TSH and thyroglobulin, because clinical decisions often rely on these values. Participation in external quality assessment programs is also recommended to maintain test accuracy and comparability. The guidelines state that thyroglobulin should always be measured alongside anti-thyroglobulin antibodies because these antibodies can interfere with thyroglobulin measurements. Laboratories should also establish protocols to detect analytical interferences caused by substances such as hemolysis, icterus, lipemia, heterophilic antibodies, biotin, or certain medications.

The postanalytical phase focuses on result interpretation and reporting. Laboratories should use method-specific and age-specific reference intervals. Special reference intervals are recommended for children, elderly individuals, and pregnant women. Trimester-specific reference intervals should ideally be used for pregnant patients. Reports should clearly state the analytical method used, because results from different immunoassay platforms may not be directly comparable. The use of standardised units and clearly defined test and method abbreviations is also recommended. For patients with differentiated thyroid cancer, laboratories should include specific thyroglobulin cut-off values to assist clinicians in monitoring the risk of recurrence.

Overall, these recommendations aim to standardise thyroid function testing practices, improve the reliability and comparability of laboratory results, and enhance communication between laboratories and clinicians. By harmonising procedures across preanalytical, analytical, and postanalytical phases, the guidelines seek to improve diagnostic accuracy and ultimately support better patient care in thyroid disease management.

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WG-3 Symposium of the Working group for tumor markers

Tumour markers: national recommendations of the Croatian Society of Medical Biochemistry and Laboratory Medicine

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Cancer remains one of the leading causes of morbidity and mortality worldwide and in Croatia, with incidence expected to continue increasing due to population ageing, lifestyle changes, and growing exposure to environmental and behavioral risk factors. Tumour markers play an important role in contemporary oncology as biological indicators associated with the presence, progression, and recurrence of malignant diseases. Although they are generally unsuitable for population-based screening of asymptomatic individuals, with the exception of selected clinical settings such as individualized prostate-specific antigen testing, they provide substantial clinical value in monitoring treatment response, detecting disease recurrence, assessing prognosis, and supporting patient follow-up. These national recommendations of the Croatian Society of Medical Biochemistry and Laboratory Medicine were developed to harmonize laboratory practice and provide comprehensive guidance on the measurement, analytical performance, interpretation, and clinical application of tumour markers. The recommendations encompass the entire laboratory testing process, including pre-analytical, analytical, and postanalytical phases, with particular emphasis on quality assurance, standardization, and harmonization of laboratory procedures. They provide practical guidance regarding patient preparation, sample collection, transport, storage conditions, analyte stability, and specific requirements for individual tumour markers. The analytical section focuses on modern immunochemical methods, including chemiluminescent immunoassay, chemiluminescent microparticle immunoassay, and electrochemiluminescence immunoassay technologies, while addressing standardization, harmonization, and method verification according to HRN EN ISO 15189 and CLSI recommendations. Particular attention is given to the recognition and management of analytical interferences such as biotin, heterophilic and human anti-animal antibodies, the high-dose hook effect, and radiological contrast media, all of which may significantly affect analytical accuracy and clinical interpretation. Practical algorithms for identifying and resolving these interferences are included. The recommendations also emphasize the importance of internal and external quality assurance, verification of analytical methods, biological variation, and appropriate interpretation of laboratory findings within the overall clinical context. Factors influencing tumour marker concentrations, including renal and hepatic dysfunction, menopausal status, pregnancy, smoking, physical activity, and diagnostic or therapeutic interventions, are comprehensively reviewed. Furthermore, the document proposes standardized interpretative comments that laboratories may include in patient reports to improve communication with clinicians and reduce the risk of misinterpretation. A dedicated section summarizes current international recommendations issued by the American Society of Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), the European Group on Tumor Markers (EGTM), and the National Comprehensive Cancer Network (NCCN) regarding

the rational use of tumour markers in the management of the most common solid malignancies, including colorectal, breast, lung, prostate, and gynecologic cancers. Clear recommendations are provided regarding their appropriate use in screening, diagnosis, prognostic assessment, treatment monitoring, and early detection of recurrence, while consistently emphasizing that tumour marker results should never be interpreted in isolation but always together with clinical findings, imaging studies, and histopathological evaluation. Although no currently available tumour marker fulfils all characteristics of an ideal biomarker, their appropriate and standardized application, combined with molecular biomarkers, TNM staging, and multidisciplinary clinical decision-making, represents an essential component of modern personalized oncology. These recommendations establish a national framework for harmonizing laboratory practice, improving analytical quality and comparability, facilitating evidence-based clinical interpretation, and ultimately contributing to improved care of patients with malignant diseases in Croatia.

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Poster abstracts

A Hematology

A-01

Is elevated MCHC in hyponatremia analyzer-dependent? A multicentric study

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Introduction: Elevated mean corpuscular hemoglobin concentration (MCHC) can be present in patients with hyponatremia; however, most published evidence pertains to Sysmex hematology analyzers (HAs). We aimed to evaluate this interference on different HAs and provide a practical solution to this issue.

Materials and methods: A total of 124 hyponatremic patients were included. Complete blood count (CBC) measurements were performed on Sysmex XN-1000 (University Hospital Sveti Duh, N = 68; University Hospital Centre (UHC) Zagreb, N = 20) and Beckman Coulter DxH 900 HAs (UHC Sestre milosrdnice, N = 36). After initial measurement, CBC samples were diluted with analyzer-specific diluent (1:2) and re-analyzed after 1 and 2 minutes. Normonatremic MCHC and mean corpuscular volume (MCV) were recorded for comparison after dilution. Results for each analyzer were compared using the Friedman paired test, with $P < 0.05$ considered statistically significant. Clinical significance was evaluated by the minimal allowed bias defined within the European Federation of Clinical Chemistry and Laboratory Medicine (EFCLM) biological variation database: 0.6% for MCHC and 1.5% for MCV.

Results: Neither normonatremic MCHC (MCHC_{normo}) nor hyponatremic MCHC (MCHC_{hyponatremic}) differed between samples measured on the Beckman DxH 900 ($P = 0.134$). MCHC_{hyponatremic} did not correlate with low sodium concentrations ($r = 0.11$, $P = 0.533$). However, a significant negative correlation between MCHC_{hyponatremic} and sodium in samples measured on Sysmex XN-1000 ($r = -0.27$, $P = 0.011$) was observed. After dilution of hyponatremic CBC samples, MCHC measured in the first minute differed significantly from both MCHC_{normo} and MCHC_{hyponatremic} ($P < 0.001$); however, MCHC measured in the second minute did not differ from MCHC_{normo}, indicating successful resolution. The average bias between MCHC_{normo} and the MCHC measured 2 minutes after dilution was insignificant (0.47%), being within the allowed bias. MCV also showed bias within the EFCLM criteria (-1.35%).

Conclusion: Beckman Coulter DxH measurements were not affected by low sodium concentrations, providing reliable MCHC results. For reliable MCHC measurements on Sysmex HAs, we propose dilution of CBC samples (1:2) using analyzer-specific diluent and re-analysis after 2-minutes incubation.

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A-02

The difference in platelet count between capillary and venous blood samples – a multicentric study

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Introduction: Capillary platelet count is considered lower compared to a venous sample; however, the results are inconclusive. To explore possible platelet count methodologies based on observed differences in the literature, we aimed to compare capillary and venous platelet counts in a multicenter study employing four different hematology analyzers.

Materials and methods: The study was conducted in four large clinical hospitals in Croatia and included the Beckman Coulter DxH 900 (impedance), Siemens Advia 2120i (optical method), and Mindray BC-6200 and Sysmex XN-1000 (both impedance and optical platelet counting methods). A total of 300 paired capillary and venous samples were collected from both inpatients and outpatients and analyzed immediately. To minimize the influence of the preanalytical phase, all four centres shared the same lot of capillary and venous K₂EDTA tubes (Beckton Dickinson, Franklin Lakes, USA). Results for each analyzer were compared using the Wilcoxon paired test (MedCalc Statistical Software v. 23.4.9, Ostend, Belgium), with $P < 0.05$ considered statistically significant.

Results: Platelet counts ranged from 124 to 597x10⁹/L. The impedance method showed significantly lower platelet counts in capillary samples: $P < 0.001$ and $P < 0.001$ for DxH 900 and XN-1000, respectively. Although platelet counts measured in capillary samples on BC-6200 were slightly lower, the difference was not statistically significant ($P = 0.093$). The optical method was not affected by sample type on Advia 2120i ($P = 0.509$) or BC-6200 ($P = 0.612$); however, platelet counts measured on XN-1000 remained significantly lower in capillary samples even when using the optical method ($P < 0.001$).

Conclusion: The effect of sample type (capillary vs venous) on platelet measurement appears to be analyzer- and method-dependent. While impedance methods seem more susceptible to sample-related differences than optical methods, significant variation between capillary and venous samples was observed on the Sysmex XN-1000 with both measurement principles.

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A-03

Analytical verification of immature granulocytes on the Sysmex XN 1000 analyzer

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Introduction: Immature granulocytes (IG) represent a clinically relevant parameter in evaluation of severe infections, inflammatory conditions and myeloproliferative disorders. Presence of IG in peripheral blood reflects increased bone marrow activity. Determination of IG (promyelocytes, myelocytes and metamyelocytes, but not band neutrophils) on automated hematology analyzers represents easily accessible parameter as part of an automated complete blood count. In accordance with current guidelines, automated IG measurement requires analytical verification before routine use. The aim of this study was to perform analytical verification of absolute and relative IG count ($10^9/L$, %) on the Sysmex XN-1000 hematology analyzer.

Materials and methods: Repeatability, within-laboratory precision, and measurement uncertainty were assessed using three levels of manufacturer's control material over five consecutive days in quintuplicate. Additionally, repeatability was evaluated using two patient samples with mildly and markedly elevated IG values, each analyzed in 20 replicates. Method comparison was conducted using 40 samples with normal and elevated IG values, with peripheral blood smears examined by light microscopy. Verification of reference intervals included IG determination in 20 apparently healthy individuals.

Results: Repeatability (2.3-4.0%), within-laboratory precision (3.6-4.0%), and measurement uncertainty (7.2-7.9%) were within the manufacturer's acceptance criteria (25-30%). Repeatability assessed in two patient samples with IG values of 3% and 18% met the predefined manufacturer's criterion of 30%, with coefficients of variation of 7.6 and 4.6%, respectively. Bland-Altman analysis demonstrated no statistically significant constant or proportional bias. Passing-Bablok regression analysis indicated the presence of statistically significant proportional bias ($y = 0.32 (-0.40 \text{ to } 1.07) + 0.77 (0.63 \text{ to } 0.94) x$) which was not clinically significant. Verification of manufacturer's reference intervals was considered acceptable because 18 out of 20 individuals fulfilled the criteria.

Conclusion: Automated determination of IG parameter on Sysmex XN-1000 analyzer demonstrated acceptable precision and measurement uncertainty and showed good agreement with light microscopy method, supporting its use in routine clinical practice.

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A-04

Analytical traps in hematological monitoring of a patient on antituberculosis therapy: a case of EDTA-induced pseudothrombocytopenia

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Introduction: Laboratory monitoring of hematological parameters during the initiation of antituberculosis therapy is critical due to the risk of drug-induced immune thrombocytopenia (DITP). Although DITP is most often associated with rifampicin, isoniazid has also been described as a rare but potential cause of severe immune-mediated platelet depletion. Differential diagnosis of thrombocytopenia in complex patients is further complicated by the possible occurrence of EDTA-induced pseudothrombocytopenia, an *in vitro* artefact caused by platelet agglutination, which can lead to the misinterpretation of drug-related myelotoxicity.

Materials and methods: A 65-year-old male patient with ankylosing spondylitis, previously treated with biological therapy (infliximab) was hospitalized for the management of disseminated tuberculosis. During the initiation of specific antituberculosis therapy (isoniazid, ethambutol), complete blood count (CBC) on automated hematology analyzer (Sysmex XN1000, Kobe, Japan) and microscopic verification of the peripheral blood smear were performed.

Results: The initial platelet count before specific antituberculosis treatment was $197 \times 10^9/L$. Following the introduction of isoniazid and ethambutol, a sharp decline to $74 \times 10^9/L$ was recorded, clinically interpreted as DITP. Follow-up analysis showed a further platelet decrease to $48 \times 10^9/L$ in EDTA-anticoagulated sample, accompanied by a "platelet clumps" analyzer flag. Microscopic examination of the peripheral blood smear confirmed the presence of numerous platelet aggregates. CBC analysis using an alternative anticoagulant (sodium citrate) yielded a significantly higher platelet count of $111 \times 10^9/L$, confirming EDTA-induced pseudothrombocytopenia in our patient. After 15 days during which transient *in vitro* platelet agglutination was present, platelet counts in EDTA-anticoagulated samples stabilized at approximately $150 \times 10^9/L$ in multiple consecutive measurements.

Conclusion: This case highlights the importance of recognizing analytical traps in complex patients with multiorgan pathology. Microscopic examination of the peripheral blood smear remains the gold standard in differentiating artifacts from true pathological conditions. Timely recognition of such analytical traps is essential to prevent unnecessary modifications of essential antituberculosis therapy and to ensure reliable and accurate laboratory results.

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A-05

Verification of the immature platelet fraction (IPF) on the Sysmex XN-1000 hematology analyzerIvana Vuga¹, Franciska Tomić^{1,2}, Nives Benček¹, Roman Mihić¹, Jasna Obuljen¹, Marija Razum¹, Sandra Margetić^{1,2}¹Department of Clinical Chemistry, University Hospital Center Sestre Milosrdnice, Zagreb, Croatia²School of Medicine, Catholic University of Croatia, Zagreb, Croatia

Introduction: The immature platelet fraction (IPF) is a newer hematological parameter that reflects the thrombopoietic activity of the bone marrow by quantifying circulating platelets with high messenger RNA (mRNA) content, analogous to the reticulocytes in erythropoiesis. Advanced fluorescent flow cytometry in the dedicated platelet-fluorescence (PLT-F) channel enables reliable detection of IPF. It is a useful parameter for differential diagnosis of thrombocytopenia according to causes *i.e.* between hyperdestructive/hyper-regenerative and hypoproliferative conditions, as well as for early prediction of platelet recovery following chemotherapy. The aim of this study was to verify the analytical performance of IPF measured in the PLT-F channel on the Sysmex XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan).

Materials and methods: Reproducibility, within-laboratory precision and measurement uncertainty were assessed using commercial control materials (XN-CHECK, Sysmex) at three levels (L1, L2, L3) over five consecutive days (N = 25) in quintuplicate. Repeatability was evaluated using two patient samples with normal (4.6%) and elevated (29.5%) IPF values, each analyzed in 20 replicates. Verification of the reference interval declared by the manufacturer (1.2–8.9%) was performed in 20 apparently healthy adults.

Results: Reproducibility, within-laboratory precision and measurement uncertainty were all within the acceptance criteria according to the manufacturer (25%), with coefficients of variations (CVs) 2.7%, 3.2% and 6.4% (L1); 3.0%, 4.2% and 8.4% (L2); and 3.8%, 3.8% and 7.6% (L3). Repeatability assessed in two patient samples also met predefined manufacturer's criterion of 25%, with CVs of 2.5% and 5.2%, respectively. Verification of manufacturer's reference intervals confirmed applicability to the local adult population, since 18 out of 20 individuals fell within the declared limits.

Conclusion: Verification procedure confirmed that IPF parameter on the Sysmex XN-1000 demonstrates acceptable analytical characteristics. Therefore, its implementation in routine laboratory practice could contribute to the improvement in clinical decision-making in patients with different causes of thrombocytopenia as well as to the timely monitoring of platelet recovery.

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A-06

Analytical verification of automated slide preparation unit Sysmex SP-50

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Introduction: The implementation of automated smearing and staining preparation has improved laboratory workflow by accelerating slide processing, standardizing smear quality and reducing manual workload. The aim was to evaluate performance of Sysmex automated slide preparation unit SP-50 (Sysmex, Kobe, Japan) compared with the manual method.

Materials and methods: Analytical verification included repeatability and comparison with the manual method. Repeatability was evaluated using three samples with increased, normal, and decreased levels of neutrophils, lymphocytes, and monocytes; 10 smears *per* sample were examined by three observers. Coefficient of variation (CV) was calculated *per* leukocyte population and observer. Method comparison was performed on 50 samples representing routine laboratory workflow, covering various patient groups and pathophysiological conditions (newborns, pediatric, intensive care, and hematological patients). Blood smears were prepared and stained simultaneously using both the manual and automated method. Three observers performed 100-leukocyte differential count on smears prepared using both methods. Bias for method comparison was calculated for each leukocyte population.

Results: Repeatability and method comparison met the criteria of the external quality assessment program (CroQALM) for leukocyte subpopulations obtained by complete blood count (neutrophils 20%, lymphocytes 20%, monocytes 30%, eosinophils and basophils 40%). Repeatability was acceptable for neutrophils (CV=3.3-14.5%), monocytes (CV=23.8-27.6%), and lymphocytes (CV=5.7-11.0%), except in a sample with extreme lymphopenia (CV=37.1-54.1%). Method comparison showed deviations outside the predefined criteria in 19/50=0.38 samples: monocytes in 13/50=0.26, lymphocytes in 5/50=0.1, and neutrophils in 1/50=0.01. All observed deviations were within reference intervals and not clinically significant, except for one highly pathological sample (monocytic leukemia). For eosinophils and basophils, all deviations were related to extremely low relative counts and were not clinically significant.

Conclusion: The analytical verification of the SP-50 automated unit confirmed its reliability as a replacement for manual blood smearing and staining. However, in case of pathological/immature cell forms, an additional manually prepared smear for microscopic differential blood count is recommended.

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A-07

Short-term stability of plasma viscosity on the Lovis 2000 M/ME viscometer

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Introduction: Plasma viscosity is an important hemorheological parameter in microcirculation regulation. Changes in viscosity often reflect pathological processes, including inflammatory and metabolic disorders. Stability of plasma samples is crucial for accurate measurement of viscosity in these conditions. However, measured results can be influenced by the method used, type of anticoagulant, and storage conditions. The aim was to investigate short-term stability of plasma viscosity in K₃EDTA and 3.2% sodium citrate tubes.

Materials and methods: Plasma samples were collected from 17 healthy individuals into K₃EDTA and 3.2% sodium citrate tubes. Plasma viscosity was measured using a Lovis 2000 M/ME viscometer (Anton Paar, Austria), within 4 hours of collection and after 10 days of storage at – 20 °C. Statistical analysis was performed using MedCalc statistical software (version 14.8.1, Ostend, Belgium). Results were presented as average, standard deviation (SD) and coefficient of variation (CV%). According to the guidelines from the EFLM Working group for the Preanalytical Phase, percentage difference (PD%) was calculated between the baseline and endpoint result.

Results: The average plasma viscosity values were as follows: K₃EDTA (room temperature, 4 h): 1.38 ± 0.06 mPa·s (CV = 4.50%), K₃EDTA (– 20 °C, 10 days): 1.34 ± 0.10 mPa·s (CV = 7.09%), sodium citrate (room temperature, 4 h): 1.29 ± 0.05 mPa·s (CV = 4.24%), and sodium citrate (– 20 °C, 10 days): 1.25 ± 0.05 mPa·s (CV = 4.36%). PD% for K₃EDTA was – 3.30%, and for sodium citrate – 3.51%.

Conclusion: Plasma viscosity showed a slight decrease after storage at – 20 °C for 10 days compared to room temperature conditions over 4 hours for both K₃EDTA and sodium citrate samples. However, the percentage differences were small (– 3.30% for K₃EDTA and – 3.51% for sodium citrate). Changes within 10% are considered expected variability so our findings suggest that short-term freezing does not affect plasma viscosity measurements regardless of the tube type used.

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A-08

Assessment regarding the way preanalytical sample preparation affects the measurement of G6PD activityDragana Šegulja^{1,2}, Kristina Kobasić Horvatić¹, Karla Žeravica²¹Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia²University of Applied Health Sciences, Zagreb, Zagreb, Croatia

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) activity is critical to preserving cellular redox balance due to NADPH produced in the pentose phosphate pathway. Glucose-6-phosphate dehydrogenase deficiency is an X-linked enzymatic disorder affecting cellular defense against oxidative stress and can lead to acute hemolytic anemia during oxidative challenges such as infections, ingestion of fava beans, or exposure to certain drugs, including antimalarials and sulfonamides. The aim of this study was to assess the impact of preanalytical sample preparation on G6PD measurement and, consequently, the possible improvement of the laboratory process.

Materials and methods: Preanalytical sample preparation implied preparation of hemolysates from washed erythrocytes or from whole blood. Washed erythrocytes were obtained by a three-step washing procedure with saline. In all prepared hemolysates by adding the digitonin reagent, G6PD activity was measured by photometric method (Randox Laboratories, Ireland) on the Alinity c analyzer (Abbott Laboratories, Germany). A parallel procedure in differently prepared samples was performed on 10 samples and by two laboratory professionals.

Results: The range of measured enzyme activities was 329-1188 IU/L. Differences between professionals in the same samples employed in washed erythrocytes were 43%, 38%, 37%, 8%, and 1%, while the maximum variation in analyzed whole blood samples was 6%. Differences between samples, washed erythrocytes and whole blood samples with a leukocyte count between 4.1 and $15.7 \times 10^9/L$, were 45% or lower, which is comparable with the maximal variation between professionals in protocol with washed erythrocytes.

Conclusion: Unevaluated laboratory protocol steps can result in significant analytical variation. Based on the study's findings, all patients with leukocytes $< 15.7 \times 10^9/L$ G6PD activity measurement can be processed without preparation of washed erythrocytes. This brief study ensured improved analytical variation and increased laboratory professionals' rational time consumption.

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A-09

The value of extended hematological parameters in patients with myelodysplastic syndromeIvona Piščak¹, Ivana Lapić^{1,2}¹Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia²Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia

Introduction: Myelodysplastic syndromes (MDSs) are myeloid neoplasms characterized by ineffective and dysplastic hematopoiesis, resulting in peripheral blood cytopenias. Modern hematology analyzers provide cell population data, reflecting morphological and functional properties of blood cells, which may improve diagnostic accuracy. This study aimed to evaluate the value of extended hematological parameters in distinguishing MDS from other cytopenias.

Materials and methods: This retrospective study comprised 59 patients with MDS and 50 patients with cytopenias of other etiologies, analyzed using the Sysmex XN-3100 hematology analyzer (Sysmex, Kobe, Japan). The study was conducted at the Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Croatia, between May 2025 and December 2025. In addition to standard complete blood count parameters, extended cell population data parameters of neutrophils, lymphocytes, and monocytes were evaluated. Statistical analysis was performed using Mann-Whitney U test for independent samples. Diagnostic performance and optimal cut-off values were assessed using receiver operating characteristic (ROC) curve analysis. Statistical significance was defined as $P < 0.05$.

Results: Significant differences between MDS patients and patients with other cytopenias were observed in multiple extended hematological parameters. Neutrophil-related parameters including NE-SFL ($P = 0.003$), NE-SSC ($P = 0.007$), and NE-WX ($P = 0.014$), lymphocyte parameters LY-X ($P = 0.004$), LY-Y ($P = 0.032$), and LY-Z ($P = 0.010$), and monocyte parameters MO-X ($P = 0.003$) and MO-WY ($P = 0.002$) showed statistically significant differences. Additionally, neutrophil activation parameters NEUT-RI ($P = 0.003$) and NEUT-GI ($P = 0.007$) were significantly different between the groups. ROC analysis demonstrated moderate diagnostic performance, with the highest area under the curve (AUC) observed for MO-WY (0.677), NE-SFL (0.667), and LY-X (0.660). NE-WY yielded the highest specificity (100%), while the highest sensitivity was observed for LY-Z (83.05%).

Conclusion: Extended hematological parameters derived from automated analyzers demonstrate potential as adjunctive diagnostic tools in differentiating MDS from other cytopenias. Their implementation in routine laboratory practice may support earlier recognition of MDS and improve selection of patients for further diagnostic evaluation.

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A-10

Diagnostic efficiency of automated reactive lymphocytosis grading indices in suspected infectious mononucleosis syndrome using Sysmex XN-1500 WDF research parametersRenat Mujagić, Karla Peruško, Lorena Honović

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Introduction: Reactive lymphocyte (virocyte) count is a highly reliable clinical indicator for the differential diagnosis of infectious mononucleosis syndrome (IMS), typically caused by the Epstein-Barr virus (EBV). This study evaluated novel research parameters derived from the WDF channel: lymphocyte fluorescence intensity (Ly-Y) and high- fluorescence lymphocyte count (HFLC#), as rapid hematological predictors for grading reactive lymphocytosis caused by the immune response to viral infection.

Materials and methods: This retrospective cohort study initially enrolled 135 patients (aged < 1–81 years) between January and April 2026. A subgroup of 108 patients was subcategorized into IMS (EBV VCAIgM positive, used as the gold standard discriminator) *versus* other IM-like syndromes. Complete blood counts and blood smears were performed using a Sysmex XN-1500 analyzer and an integrated SP-50 blood slide maker (Sysmex, Kobe, Japan). Cytomorphological differentiation was analyzed using a CellaVision DM 1200 (Lund, Sweden). Statistical analysis was performed using MedCalc software (version 14.8.1, Ostend, Belgium).

Results: Ly-Y demonstrated the highest diagnostic performance (ANOVA: F-ratio = 23.9; $P < 0.001$), with values (mean $\pm$ standard deviation, SD) of 77.2 ± 7.0 [ch] for the IMS (target group) *versus* 70.1 ± 8.1 [ch] for the IM-like syndrome (comparison control group). The calculated optimal cut-off value was > 72.7 [ch] ($Sn = 78.0\%$, $Sp = 69.4\%$). A logistic regression analysis showed synergistic predictive efficiency between Ly-Y ($P < 0.001$) and total lymphocyte proportion ($P < 0.002$) in distinguishing IMS, validated by *post-hoc* ROC analysis (area under curve, $AUC = 0.806$; 95% CI 0.719 to 0.876). In the overall cohort of 135 patients, significant rank intercorrelations were found between virocytes/plasmacytes and HFLC# ($rs = 0.757$; 95% CI 0.674 to 0.821) and Ly-Y ($rs = 0.685$; 95% CI 0.584 to 0.766).

Conclusion: Ly-Y and HFLC# are reliable, rapid, and real-time automated parameters that can efficiently distinguish IMS from similar IM-like syndromes. These findings suggest their appropriate implementation into routine laboratory diagnostic guidelines to enhance the diagnostic efficiency using prescreening emergency laboratory indices that may be recommended as standard operating laboratory procedure on IMS suspicion.

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A-11 (Oral presentation)**Reference intervals for hematological parameters in the umbilical cord blood samples on Advia 2021i hematological analyzer**

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Introduction: Umbilical cord blood (UCB), collected during birth, can be stored and serves as a source of hematopoietic stem cells (HSC) for transplantation or regenerative therapies. Before UCB processing and cryopreservation, a quality assessment is necessary, including a complete blood count (CBC). This study aimed to establish reliable reference intervals (RIs) for CBC parameters in UCB samples using the hematological analyzer Advia 2021i.

Materials and methods: Umbilical cord blood were collected "ex-utero" and processed in accordance with institutional standard operating procedures. Before processing, one mL of the UCB sample was taken, and CBC parameters were determined on the Advia 2021i (Siemens Healthcare, Marburg, Germany). Double-side 95% RIs between 2.5th and 97.5th percentiles for all parameters were determined using MedCalc 14.12.0 software (MedCalc Software, Ostend, Belgium).

Results: During the observed period, 596 UCB units were received from the maternity hospital. In this study only units with a volume greater than 90 millilitres (N = 264, 44%) were included. Reference intervals for UCB hematological parameters were as follows: WBC $5.4\text{--}17.8 \times 10^9/\text{L}$, RBC $2.48\text{--}4.02 \times 10^{12}/\text{L}$, HGB 90–141 g/L, HTC 0.308–0.476 L/L, PLT $122\text{--}296 \times 10^9/\text{L}$, NRBC $0\text{--}3.49 \times 10^9/\text{L}$, neutrophils 35–67%, lymphocytes 18.4–49.2%, monocytes 4.5–13.6%, eosinophils 1.1–8.1%, and basophils 0–2.1%. Significant difference was observed for WBC ($P = 0.045$), neutrophils ($P = 0.003$), and eosinophils ($P < 0.001$) by gender, where WBC and neutrophils had greater values in females, and eosinophils in males. Analyzing the results according to the type of delivery, a statistically significant difference was obtained for WBC ($P < 0.001$), RBC ($P = 0.019$), HGB ($P = 0.030$), HTC ($P = 0.021$), PLT ($P = 0.005$), neutrophils ($P = 0.004$), and lymphocytes ($P = 0.019$). All parameters were higher in vaginal delivery, except lymphocytes, which were higher in caesarean section.

Conclusion: For the first time in Croatia, RIs for hematological parameters in UCB have been determined, which ultimately contributes to a better and more precise quality assessment of HSC product in the UCB bank.

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A-12

Establishment of indirect reference intervals for platelet indices on Sysmex XN-3100 hematology system

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Introduction: Information on reference intervals (RI) for platelet indices provided by manufacturers is often limited because these intervals are usually analyzer-dependent and supported by limited published data which makes them not completely transferable to the local patient population. The aim of the present study was to establish RIs for platelet indices, *i.e.* mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR), plateletcrit (PCT) and immature platelet fraction (IPF) measured on Sysmex XN-3100 (Sysmex, Kobe, Japan) using the indirect method.

Materials and methods: The initial database comprised 99,964 complete blood count measurements that were obtained in a six-month period, from May to October 2025. Exclusion criteria were patients from hematological wards, reports with platelet counts outside the reference range and those with platelet-related flags. The final cohort included 58,572 patient results. For each parameter, 95% double-sided RIs that include the range between the 2.5th and 97.5th percentiles were determined with 90% confidence intervals (CI). Statistical analysis was performed using MedCalc, version 23.5.2 (Ostend, Belgium).

Results: For all participants (N = 58,572) calculated RI for MPV is 8.6 fL (90% CI: 8.6 to 8.6) to 11.9 fL (90% CI: 11.9 to 11.9); for PDW 8.4 fL (90% CI: 8.3 to 8.4) to 15.3 fL (90% CI: 15.3 to 15.4); for P-LCR 13.7% (90% CI: 13.6 to 13.8) to 40.2% (90% CI: 40.1 to 40.3); for PCT 0.16% (90% CI: 0.16 to 0.16) to 0.40% (90% CI: 0.40 to 0.41). For IPF (N = 389) calculated RI is 0.7% (90% CI: 0.7 to 0.9) to 9.1% (90% CI: 8.0 to 11.6).

Conclusion: This study established new RIs for platelet indices determined on Sysmex XN-3100. Their implementation into routine practice can improve interpretation of platelet results and contribute to the management of platelet disorders.

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A-13

Stability of samples used in quality assessment of complete blood count in hematopoietic stem cell products

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Introduction: Sample stability is an important factor in the whole laboratory process. Especially if the samples are difficult to obtain, such as hematopoietic stem cell (HSC) products or umbilical cord blood (UCB) units. Determination of complete blood count (CBC) parameters is part of the quality assessment of cell products. The aim of this study was to assess the stability of whole peripheral blood samples and HSC product samples for CBC parameters determination.

Materials and methods: The samples analyzed in the study were mobilized (N = 37) and non-mobilized (N = 50) whole peripheral blood, leukapheresis HSC products (N = 42), mononuclear cell (MNC) products collected for extracorporeal photopheresis (ECP) (N = 45), UCB units (N = 41), UCB buffy coat (UCB-BC) (N = 39) and bone marrow products (N = 21). Complete blood count parameters were analyzed on hematology analyzer Advia 2120i (Siemens Healthcare, Marburg, Germany). All samples were analyzed within one hour of sampling (baseline), stored in a refrigerator at + 4 °C, and re-analyzed after 24h. Statistical analysis was performed using Medcalc statistical software, version 14.12.0 (MedCalc Software, Ostend, Belgium).

Results: There was statistically significant difference between baseline measurement and measurement after 24 hours on + 4 °C for parameters in mobilized peripheral blood (Htc P = 0.004), non-mobilized peripheral blood (WBC P = 0.033, Htc P = 0.001, PLT P < 0.001, basophile percentage P = 0.001), leukapheresis products (RBC P < 0.001, Htc P < 0.001, PLT P < 0.001, lymphocyte percentage P = 0.016), MNC products for ECP (basophile percentage P = 0.001), UCB units (PLT P < 0.001, basophile percentage P < 0.001) and UCB-BC (ERC P = 0.032, neutrophile percentage P = 0.001, basophile percentage P < 0.001). No statistically significant difference was observed for the stability of parameters in bone marrow.

Conclusion: Results showed that the analyzed sample types used in cell therapy, except bone marrow, were not stable after 24h of storage in refrigerator at + 4 °C. Therefore, it is recommended that CBC parameters not be determined after 24h storage under the conditions tested in this study for the specified sample types.

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A-14

Comparison of erythrocyte sedimentation rate (ESR) measured on VISION Pro with the Westergren reference method

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Introduction: The manual Westergren method is the established reference for erythrocyte sedimentation rate (ESR) determination, typically requiring one hour and specific citrate tubes. In contrast, the Vision Pro analyzer (Shenzhen YHLO Biotech, Shenzhen, China) provides automated ESR measurements from primary EDTA tubes in 20 minutes by monitoring light transmission changes. This study aimed to evaluate the comparability between the manual Westergren method and the automated Vision Pro analyzer.

Materials and methods: Blood samples from 100 outpatients with hematocrit levels > 0.35 were analyzed. Manual ESR was performed using VACUETTE ESR tubes (Greiner Bio-One, Kremsmünster, Austria) with pipette Vacupeta (LT Burnik, Vodice, Slovenia) while automated ESR on the VISION Pro was determined in 'cycle mode' using VACUETTE K₃EDTA tubes (Greiner Bio-One, Kremsmünster, Austria). Results from the Vision Pro were analyzed without temperature correction.

Results: Passing-Bablok regression yielded the equation $y = 2.0 + 1.0 \times$ (95% CI intercept: 0.56 to 2.06; slope: 0.96 to 1.11). Bland-Altman analysis showed a mean bias of -2.11 (95% CI: -3.31 to 0.91; $P < 0.005$). Pearson correlation was excellent ($r = 0.938$; $P < 0.001$), while Spearman's coefficient was 0.864 ($P < 0.001$). The Cusum test indicated a significant deviation from linearity ($P = 0.04$), justifying the use of Passing-Bablok regression over ordinary linear models.

Conclusion: Despite a slight deviation from linearity across the measured range, Passing-Bablok and Bland-Altman analyses confirmed a systematic but non-proportional difference. This constant bias is considered acceptable as it is negligible at clinically significant values. The VISION Pro analyzer is a satisfactory replacement for the manual Westergren method, though further evaluation with a larger sample size, focusing on pathological values, is recommended before full-scale routine implementation.

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A-15

Comparison of two methods for schistocyte quantification on the automated hematology system Sysmex XN-3100

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Introduction: The automated hematology system Sysmex XN-3100 (Sysmex Corp., Kobe, Japan) enables automated counting of fragmented red blood cells (FRC) and additionally, detection and estimation of the schistocyte count in peripheral blood smears on the digital morphology analyzer DI-60 (SHISTO-DI). This study hypothesized that there is a correlation between FRC and SHISTO-DI. This study aimed to compare the results obtained by using two methods for schistocyte quantification, both available on the system.

Materials and methods: The study included 84 EDTA blood samples from 52 patients, referred to the hematology laboratory for routine complete blood count (CBC) analysis. For each sample, automated FRC count as part of the CBC was obtained by fluorescence flow cytometry in the reticulocyte channel on the XN-1000 analyzer. Schistocyte count in peripheral blood smears, prepared and stained using the automated slide marker and stainer SP-50, was estimated using a locally adapted SHISTO-DI method on DI-60. The smear area corresponding to approximately 8.5 high-power fields was examined and the schistocytes were counted. Results were expressed as percentages for both methods. Statistical analysis was performed using MedCalc Software, v23.4.8 (Ostend, Belgium), by applying Passing-Bablok and Bland-Altman analysis.

Results: Obtained median FRC value was 0.44% (95%CI: 0.34 to 0.60; interquartile range, IQR: 0.10-0.99), whereas median SHISTO-DI value was 0.35% (95%CI: 0.24 to 0.63; IQR: 0.12-1.65). Method comparison study revealed the following regression equation: $y = 0.20$ (95%CI: 0.004 to 0.284) + 0.56 (95%CI: 0.36 to 0.86) x , with a Spearman rank correlation coefficient of 0.219 ($P = 0.045$). Bland-Altman analysis yielded the median difference of 0.05% (95%CI: - 2.70 to 4.64) between the two methods.

Conclusion: Although the obtained results indicated both constant and proportional differences between FRC and SHISTO-DI, a weak positive correlation was still confirmed. The possible explanation of the weak correlation is completely different technology applied in these methods for schistocyte quantification.

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A-16

Evaluation of multiparameter flow cytometry as a diagnostic tool for multiple myeloma: a comparison with bone marrow biopsy

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Introduction: Alongside the gold-standard bone marrow biopsy (BMB), multiparameter flow cytometry (MFC) may improve differentiation between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM). This study evaluated the diagnostic performance of MFC compared with BMB and the final clinical diagnosis.

Materials and methods: A retrospective analysis was performed on patients with suspected MGUS/MM treated at UHC Zagreb in 2025/2026. Multiparameter flow cytometry criteria for MM was defined as a proportion of aberrant plasma cells (aPC) within the bone marrow plasma cell compartment (aPC/BMPC) > 95%. Bone marrow biopsy criteria for MM was defined as bone marrow aPC/BMPC $\geq$ 60% or a lower value according to clinical guidelines when other criteria are met. Statistical analysis was performed using MedCalc Software Ltd (Ostend, Belgium). Diagnostic sensitivity (Sn), specificity (Sp) and accuracy were calculated for both methods and McNemar test was used for comparison of the methods. We also performed receiver operating characteristic (ROC) analysis for the MFC.

Results: Altogether, 35 patients (median age 71 (52-82); males = 11, females = 24) with reliable FC and BMB reports were included. Twelve patients were classified as MM by MFC criteria, and 9 by BMB criteria. The diagnostic testing performance metrics for the MFC were: Sn = 71.4%, Sp = 90.5%, diagnostic accuracy = 82.9%; for the BMB: Sn = 64.3%, Sp = 100%, diagnostic accuracy = 85.7%. The McNemar test was performed and no statistically significant difference between the diagnostic performance of examined methods was found. ROC analysis results for MFC were: area under curve (AUC) = 0.765, optimal cut-off > 90%; 95% CI: 63.7 to 97.0.

Conclusion: Multiparameter flow cytometry demonstrated higher sensitivity, identifying more true MM cases, whereas BMB show higher specificity with no false positives. Bone marrow biopsy obtained slightly higher overall accuracy and specificity, but the difference is too small in relation to the sample size to draw any statistical conclusions about the superiority of either method. ROC analysis showed moderate diagnostic performance of MFC. Novel criteria could increase the diagnostic impact of MFC for the differential diagnosis of MGUS/MM.

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A-17 (Oral presentation)**Investigating the stability of third party quality controls across hematology analyzers**

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Introduction: The ISO 15189:2022 and CLSI-C24 guidelines strongly recommend using third-party internal quality controls to ensure unbiased performance assessment. The study aimed to evaluate the stability and analytical performance of Randox's Acusera Hematology Controls on Sysmex XN1000 (A1) and XN550 (A2) analyzers.

Material and methods: The complete blood count (CBC) was measured for 14 days at three control levels (L1, L2, L3) on each analyzer, matching the claimed 14-day open-vial stability at 2-8 °C. Both analyzers (Sysmex Kobe, Japan) use hydrodynamically-focused impedance for red blood cells, platelets (PLTs), and their associated parameters; cyanide-free SLS for hemoglobin, and fluorescence flow cytometry for leukocyte count with 5-part differential (DIFF), distinguishing neutrophils (Ne), lymphocytes (Ly), monocytes (Mo), eosinophils (Eo) and basophils (Ba). Assessment of nucleated red blood cells (NRBCs) is available only on the A1. Data were analyzed using Microsoft Excel 2016 (Microsoft, Redmond, USA). Coefficient of variation (CV) and bias (B) were calculated and compared against the manufacturer's and CROQALM's allowable limits, respectively.

Results: Over the 14 days, total CV and B for all CBC parameters on A1 at all levels met allowable limits, except for Mo% at L1 (CV: 34.6%; B: - 58.6%). NRBCs remained undetected across all levels. From day 9, PLT at L2 declined, with an unacceptable bias ranging from - 16.4% to - 20.5%. Considering A2, limits were exceeded for Ly (CV: - 9.6%; B: - 31.6%) at L1. Unacceptable bias was recorded for Mo% from the very first day, with overall mean biases of - 56.8% for L2 and - 59.4% for L3. PLTs at L2 declined from day 10 onward, with an unacceptable bias ranging from - 16.8% to - 20.9%.

Conclusion: Overall, the 14-day stability claim cannot be fully supported, as several parameters did not meet expected analytical performance, and the third-party controls did not demonstrate sufficient reliability for routine use at the time.

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A-18

Diagnostic challenges in T/Myeloid mixed phenotype acute leukemia: the value of multiparametric flow cytometry integrated with cytogenetic and molecular findings

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Introduction: Mixed phenotype acute leukemia (MPAL) is a rare and biologically heterogeneous subtype of acute leukemia characterized by the expression of lineage-defining markers from more than one hematopoietic lineage. Due to its rarity and immunophenotypic complexity, establishing the diagnosis may be challenging. Multiparametric flow cytometry (MFC) plays a central role in differentiating MPAL from acute leukemias with aberrant antigen expression and in guiding subsequent therapeutic decisions.

Materials and methods: We report the case of a 22 year old male presented in February 2025 with prolonged fever, pancytopenia and mild splenomegaly. Initial evaluation in an outside institution suggested acute myeloid leukemia (AML); however, comprehensive diagnostic workup at our institution established the diagnosis of T/Myeloid MPAL according to World Health Organization (WHO) criteria.

Results: Multiparametric flow cytometry analysis demonstrated a blast population co-expressing immature T-cell lineage markers (cytoplasmic CD3, CD7 and terminal deoxynucleotidyl transferase (TdT)) together with myeloid markers (myeloperoxidase, CD117 and CD33), with aberrant CD56 expression and absent HLA-DR expression. Cytogenetic analysis revealed a complex karyotype with structural abnormalities involving chromosomes 6q, 7p, 11q and 13q. Fluorescence in situ hybridization detected KMT2A(MLL) deletion and deletion of 13q14. Molecular studies identified a PTEN frameshift mutation (p.Leu247GlyfsTer7), while T-cell receptor gamma clonality testing demonstrated clonal rearrangement. The patient received induction and consolidation treatment according to the CLIA-Venetoclax protocol, followed by allogeneic hematopoietic stem cell transplantation from a haploidentical donor. Complete remission with measurable residual disease negativity and complete donor chimerism was achieved; however, disease relapse subsequently occurred despite donor lymphocyte infusion (DLI). The patient ultimately died due to progressive disease.

Conclusion: This case highlights the diagnostic value of MFC in the recognition of MPAL and demonstrates the importance of integrating immunophenotypic, cytogenetic and molecular findings for accurate disease classification and clinical management.

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A-19

5-DIFF vs. 3-DIFF: comparison of differential blood count on Sysmex XS-1000i and Microsemi CRP LC-767G hematology analyzerTina Brenčić¹, Katarina Jedrejčić²¹Medical Biochemistry Laboratory, Istrian Health Center, Labin, Croatia²Medical Biochemistry Laboratory, Istrian Health Center, Pazin, Croatia

Introduction: Hematology analyzer Sysmex XS-1000i (Sysmex Corporation, Kobe, Japan) with 5-part differential complete blood count (CBC) was replaced with Microsemi CRP LC-667G (Horiba Ltd, Kyoto, Japan) with 3-part differential CBC in our laboratory. Considering different technologies for differential blood count (DBC) determination, this study aimed to compare DBC on these analyzers.

Materials and methods: The study included 57 K₃EDTA blood samples (Greiner Bio-One, Kremsmünster, Austria) in which CBC was determined on both analyzers. The differences in hematology parameters were assessed by the Mann-Whitney test and Bland-Altman analysis. The neutrophil count (NEUT) on Sysmex was compared with the granulocyte count on Microsemi. Monocyte count (MONO) on Microsemi is supposed to include monocytes, eosinophils and basophils (MONO+EOS+BASO), so it was compared with MONO and additionally with the sum of these cell populations on Sysmex. Average bias (%) was compared to the allowed bias according to the CROQALM acceptance criteria (WBC $\pm$ 15%; NEUT $\pm$ 20%, LYMP $\pm$ 20%, MONO $\pm$ 30%). Finally, the presence of DBC related flags on each analyzer was compared. Statistical analysis was performed in MedCalc v23.5.5 (MedCalc Software Ltd, Ostend, Belgium) with $P < 0.05$ considered statistically significant.

Results: There were no statistically significant difference in WBC, NEUT and LYMP obtained by two analyzers; $P = 0.946$, $P = 0.411$, $P = 0.269$, respectively. There was statistically significant difference in MONO whether we consider only the MONO ($P < 0.001$) or MONO+EOS+BASO ($P = 0.014$) on Sysmex. Average biases were - 0.83 (95% confidence interval (CI): - 2.83 to 1.16)% for WBC, 14.72 (95%CI: - 19.80 to - 9.64)% for NEUT, 8.68 (95%CI: 3.99 to 13.37)% for LYMP, 42.53 (95%CI: 33.69 to 51.36)% for MONO and - 21.70 (95%CI: - 29.36 to - 14.02)% for MONO+EOS+BASO on Sysmex, indicating significant constant bias between MONO on tested analyzers. In 14% of samples (8/57), the DBC flag was absent on Microsemi, but present on Sysmex.

Conclusion: Due to the limitations of 3-differential technology for DBC, suspicious MONO results on Microsemi should be used with caution and checked by microscopic examination of peripheral blood smear or a 5-differential hematology analyzer.

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A20

Precision of differential blood count performed using the digital cell morphology analyzer Sysmex DI-60

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Introduction: Digital peripheral blood cell morphology analysis represents a modern approach to determine the differential blood count (DBC). This study aimed to evaluate the precision of DBC analysis, both for normal and pathological DBCs, on Sysmex DI-60 digital cell imaging analyzer (Sysmex Corp., Kobe, Japan).

Materials and methods: Seven peripheral blood smears were analyzed; three from routine EDTA samples, stained using May-Grünwald-Giemsa, on Sysmex SP-50 analyzer (one without pathology, two with acute leukemia), and four provided by the German Reference Institute for Bioanalytics external quality assessment program (two without pathology, one with chronic myeloid leukemia, one with viral infection). The evaluation of repeatability and intermediate precision according to CLSI guideline EP15-A3, included analysis of DBCs on 100 leukocytes (five replicates during five consecutive days). Subsequently, coefficients of variation (CVs) were calculated.

Results: Obtained CVs for repeatability were as follows: for segmented and non-segmented granulocytes, lymphocytes, and monocytes 2.2%-353.6% (smears without pathology), 8.5%-48.6% (acute leukemias), 3.2%-6.2% (chronic myeloid leukemia), 3.5%-61.8% (viral infection); for eosinophilic and basophilic granulocytes and immature cells 2.9%-500.0% (smears without pathology), 0.2%-136.0% (acute leukemias), 11.2%-77.2% (chronic myeloid leukemia) and 7.3%-500.0% (viral infection). Obtained intermediate precision CVs were as follows: for segmented and non-segmented granulocytes, lymphocytes, and monocytes 0.6%-136.9% (smears without pathology), 2.8%-30.6% (acute leukemias), 3.8%-11.4% (chronic myeloid leukemia), 2.1%-51.3% (viral infection); for eosinophilic and basophilic granulocytes and immature cells 1.1%-223.6% (smears without pathology), 4.1%-85.3% (acute leukemias), 3.6%-23.9% (chronic myeloid leukemia), 6.9%-223.6% (viral infection).

Conclusion: Obtained CVs are comparable to literature data for precision of DBCs performed by light microscopy. Very high CVs, albeit not clinically significant, for both precisions were encountered for leukocyte subpopulations with very low proportions in the DBCs in all smears, regardless the presence of pathology. Results heterogeneity is likely due to insufficiently defined morphological criteria, subjective interpretation and individual variability in their application.

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A-21

Can automated cerebrospinal fluid analysis on Sysmex XN-1500 reliably replace manual methods?

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Introduction: Cerebrospinal fluid (CSF) leukocyte analysis is performed by counting in a Fuchs–Rosenthal chamber and morphological evaluation of cytocentrifuge-prepared and Pappenheim-stained smears. Due to time-consuming nature of the method, there is an increasing demand for the automation and acceleration of the process. This study aimed to evaluate the comparability of leukocyte counting and differentiation on the hematology analyzer with the manual methods.

Materials and methods: A total of 129 CSF samples were analyzed, with leukocyte count from 5 to 16,000 $\times 10^6/L$. Samples were measured using Sysmex XN-1500 (Sysmex, Kobe, Japan) in body fluid mode, providing leukocyte count and percentage (%) of polymorphonuclear and mononuclear cells. Manual analysis included cell counting in Fuchs–Rosenthal chamber and microscopic differentiation. Statistical analysis was performed in MedCalc software using Bland-Altman analysis, Passing-Bablok regression, and Cusum linearity testing.

Results: Bland-Altman analysis for leukocytes showed relative bias of - 7.68% (95% confidence interval (CI): -14.07 to -1.30). Passing–Bablok regression showed no significant constant or proportional error (intercept - 0.29; 95% CI: - 4.80 to 3.11, slope 1.03; 95% CI: 0.99 to 1.06), and no significant deviation from linearity ($P = 0.94$). Bland-Altman polymorphonuclear analysis showed an absolute bias of 0.42% (95% CI: - 0.54 to 1.37). Passing–Bablok regression showed no significant constant or proportional error (intercept 0.03; 95% CI: - 0.002 to 0.30, slope 1.00; 95% CI: 0.98 to 1.01), with no deviation from linearity ($P = 0.40$). Mononuclear cells showed an absolute bias of - 0.49% (95% CI: - 1.39 to 0.41). Regression analysis indicated no significant constant or proportional error, (intercept 0.20; 95% CI: - 1.13 to 2.09, slope 1.00; 95% CI: 0.98 to 1.01) and no deviation from linearity ($P = 0.40$).

Conclusion: Based on the comparative analysis, Sysmex XN-1500 demonstrated acceptable agreement with the reference method, confirming that automated leukocyte, polymorphonuclear and mononuclear count can reliably replace time consuming manual methods and significantly shorten turnaround time.

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B Hemostasis

B-01

The M-protein trap: discrepant results between optical and impedance platelet aggregometry

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Introduction: Monoclonal proteins (M-proteins) can interfere with various laboratory assays, potentially leading to misleading clinical interpretation. We report a case of discrepant platelet aggregation results, as assessed by light transmission aggregometry (LTA) and impedance aggregometry (IA), in a patient with an extremely high M-protein concentration. A 56-year-old female was referred to a transfusion specialist following an episode of unusual ocular bleeding. A slightly prolonged prothrombin time (0.57-0.66) was observed during initial screening, and further diagnostics were subsequently carried out through platelet aggregation.

Materials and methods: Platelet aggregation was assessed by LTA (Sysmex CS-2500, Siemens Healthineers) using ADP, arachidonic acid, collagen, epinephrine, and ristocetin, and by IA (multiplate analyzer, Roche Diagnostics) with ADP and arachidonic acid. Venous blood was collected in 3.2% sodium citrate tubes for LTA, and in heparinized tubes for IA (BD Vacutainer, Becton Dickinson, Franklin Lakes, USA). Platelet-rich plasma and platelet-poor plasma were prepared by centrifuging citrated blood for 10 min at 95xg and 1520xg, respectively. Complete blood count was performed on a Sysmex XN-1000 analyzer.

Results: Light transmission aggregometry showed no response to any agonist, resulting in flat-line traces. The platelet count was $136 \times 10^9/L$, which is sufficient for optical aggregometry. In contrast, IA showed only moderately reduced aggregation. Subsequent biochemical analysis revealed severe hyperproteinemia with total protein levels of 170.8 g/L, while serum protein electrophoresis and immunofixation confirmed a monoclonal IgG kappa protein (IgG 105 g/L).

Conclusion: Our findings suggest that M-proteins significantly interfered with LTA, whereas IA appeared less affected. Since IA is performed in whole blood and better reflects physiological conditions, it may provide a more reliable functional assessment in cases of extreme hyperproteinemia. As previous studies have also noted M-protein interference with LTA, caution is required when interpreting aggregometry results in patients with monoclonal gammopathies, regardless of the method used.

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B-02

Influence of hemolysis on standard coagulation assays using the photo-optical method

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Introduction: Hemolysis represents a major preanalytical source of interference in coagulation testing performed on photo optical analysers. This study evaluated the impact of hemolysis on prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB) measurements using the Sysmex CS2500 (Sysmex Corporation, Kobe, Japan), which utilizes automated sample-integrity checks to identify interference. We aimed to define criteria for the acceptance or rejection of hemolysed coagulation samples.

Materials and methods: Blood samples were collected into 3.5 mL tubes containing 3.2% sodium citrate and centrifuged (2100xg/15 min). Following analysis on the Sysmex CS2500, from 56 non-hemolysed residual samples, eight pools were prepared. One aliquot was labeled as zero hemolysis-H0, while the remaining blood underwent mechanical hemolysis by being passed through a 30G insulin syringe one, two, three, and four times to obtain hemoglobin concentrations (HC) of 1 (H1), 2 (H2), 3 (H3), and 4 (H4) g/L. After centrifugation, PT, APTT, FIB, HC, and hemolysis index (HI) were determined. Hemolysis was assessed using both CS2500 (HI) and Architect c4000 (HI, HC). All measurements were performed in duplicate within 4 hours of collection. Statistical analysis was conducted using MedCalc v20.008 (MedCalc Software, Ostend, Belgium).

Results: Agreement between HI measurements obtained from the Architect c4000 and CS2500 was moderate ($\kappa = 0.585$; 95% confidence interval (CI) 0.458 to 0.712). Hemoglobin concentrations ranged from H0 = 0.03 to H4 = 4.32 g/L, covering most hemolysed samples in routine practice. A statistically significant difference was noted only for PT at H3 compared to H0 ($P = 0.026$), without clinical significance. The percentage deviations from baseline, including their 95% CI, remained within the total allowable error limits defined by the EFLM database for all assays at hemoglobin concentrations up to 4.32 g/L.

Conclusion: Analyzer CS2500 reliably detects hemolysis, but quantitative hemoglobin measurement provides a more robust basis for sample assessment. Samples with HC below 4.32 g/L are acceptable for PT, APTT, and FIB testing.

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B-03

Can citrate plasma samples be used for aPTT testing after 24 hours at + 4 °C? A comparison analysis of two coagulation analyzers

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Introduction: This study evaluated whether citrate plasma samples stored in capped primary tubes for 24 hours at + 4 °C remain suitable for activated partial thromboplastin time (aPTT) testing, using two coagulation analyzers.

Materials and methods: Ninety routine patient citrate plasma samples were analyzed within 4 hours of blood collection and after 24-hour storage at + 4 °C in capped primary tubes, by using Actin FS on a reference Sysmex CS-5100 analyzer (Sysmex Corporation, Kobe, Japan) and aPTT reagent on cobas t711 (Roche, Basel, Switzerland). Paired baseline and 24-hour results were compared using the Wilcoxon signed-rank test (MedCalc v22.007, MedCalc Software Ltd, Ostend, Belgium). Results were statistically significant at $P < 0.05$. Mean percentage bias (MPB) was compared with the Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) allowable limit of 7%.

Results: Median baseline aPTT values were 24.6 s (95% confidence interval (CI): 23.8 to 25.3; interquartile range (IQR): 22.2-26.9) on Sysmex CS-5100 and 28.1 s (95%CI: 27.3 to 29.0; IQR: 25.8-30.4) on cobas t711. After 24-hour storage, median aPTT increased to 26.5 s (95%CI: 25.6 to 27.3; IQR: 23.9-29.2) on Sysmex CS-5100 (1.9 s increase) and to 29.6 s (95%CI: 28.7 to 30.6; IQR: 27.2-32.3) on cobas t711 (1.5 s increase). For both analyzers, these differences were statistically significant ($P < 0.001$). Bias ranged from - 6.8% to 25.6% for Sysmex CS-5100, with an MPB of 8.1%, exceeding the CROQALM allowable limit of 7%. For cobas t711, bias ranged from - 8.0% to 34.9% with MPB 6.9%, which was within the CROQALM allowable limit.

Conclusion: Citrate plasma samples stored in capped primary tubes for 24 hours at + 4 °C may remain suitable for aPTT testing, depending on the reagent-analyzer system used. These findings underscore the importance of accounting for analyzer-dependent variability when assessing sample suitability after storage. Caution is warranted when interpreting results, as even differences within allowable limits may shift values from the normal to the pathological range.

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B-04

Verification of the cobas t711 coagulation analyzer in a tertiary care laboratoryAnamarija Bogić¹, Merima Čolić², Désirée Coen Herak^{1,3}, Dunja Rogić^{1,3}¹Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia²Department of Laboratory Diagnostics, Children's Hospital Zagreb, Zagreb, Croatia³Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Introduction: This study aimed to verify the performance of the cobas t711 coagulation analyzer (Roche, Basel, Switzerland).

Materials and methods: Prothrombin time (PT), INR, activated partial thromboplastin time (APTT), fibrinogen, antithrombin, D-dimer, FVIII, FXIII, and von Willebrand factor (VWF) activities were analyzed. Precision (repeatability, between-run, and within-laboratory) and bias were assessed over five days using commercial controls at two levels. The acceptance criteria for precision were the upper verification limit (UVL), calculated using the manufacturer's precision claims and the factor F (determined by degrees of freedom), and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variability criteria. For bias, Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) and EFLM criteria were used. Method comparison was performed using patient plasma samples and Sysmex CN-3000 and CS-5100 analyzers (Sysmex Corporation, Kobe, Japan), applying Passing-Bablok analysis (MedCalc v22.007, MedCalc Software Ltd, Ostend, Belgium).

Results: Prothrombin time and INR met UVL and EFLM criteria for precision, while antithrombin partially met them. Other analytes met at least the minimal EFLM criteria, except FXIII, which partially met UVL and had no defined EFLM criteria. Activated partial thromboplastin time, fibrinogen, and D-dimer met EFLM and CROQALM bias criteria. Prothrombin time and antithrombin met only CROQALM, and INR partially met CROQALM criteria. Von Willebrand factor and FVIII had no CROQALM criteria; VWF partially met while FVIII did not meet EFLM criteria. FXIII had no defined bias criteria. Method comparison study demonstrated no differences for FVIII ($N = 48$, $y = 0.48 (-0.45 - 1.21) + 0.97(0.92 - 1.03) x$, $r = 0.982(0.968 - 0.990)$) and VWF ($N = 92$, $y = 0.06(-3.22 - 3.35) + 0.98(0.93 - 1.02) x$, $r = 0.989(0.984 - 0.993)$). Constant differences were observed for antithrombin ($N = 121$, $y = -3.88(-6.70) - (-0.72) + 0.96(0.92 - 1.00) x$, $r = 0.895(0.852 - 0.925)$) and D-dimer ($N = 66$, $y = -0.34(-0.68) - (-0.12) + 0.95(0.83 - 1.02) x$, $r = 0.983(0.973 - 0.990)$). FXIII exhibited a proportional difference ($N = 39$, $y = -2.25(-6.80) - 3.15 + 0.94(0.87 - 0.99)$, $r = 0.980(0.962 - 0.989)$). Both constant and proportional differences were encountered for PT ($N = 228$, $y = 2.68(1.00 - 4.34) + 0.82(0.80 - 0.84) x$, $r = 0.986(0.982 - 0.990)$), INR ($N = 228$, $y = -0.04(-0.06) - (-0.02) + 1.06(1.04 - 1.08) x$, $r = 0.990(0.988 - 0.993)$), aPTT ($N = 316$, $y = 1.86(1.14 - 2.56) + 1.04(1.01 - 1.07) x$, $r = 0.978(0.972 - 0.982)$), and fibrinogen ($N = 109$, $y = 0.32(0.26 - 0.39) + 0.84(0.82 - 0.87) x$, $r = 0.989(0.984 - 0.993)$).

Conclusion: The cobas t711 demonstrated acceptable performance for routine coagulation testing, especially for PT/INR, aPTT, fibrinogen, and D-dimer. However, variable precision and observed constant or proportional differences require assay-specific interpretation when comparing platforms.

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B-05

Evaluation of lupus anticoagulant screening and confirmation reagents on the cobas t711 coagulation analyzer

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Introduction: This study evaluated two phospholipid-dependent diluted Russell's viper venom time (dRVVT) assays for lupus anticoagulant diagnosis, Lupus S (screening) and Lupus C (confirmation), on the cobas t711 coagulation analyzer (Roche, Basel, Switzerland).

Methods and materials: Analytical verification included precision assessment, establishment of reference intervals (RI), and method comparison. Precision (repeatability, between-run, within-laboratory) was assessed over five days with commercial controls at two levels. Acceptance criteria were upper verification limits (UVL), based on the manufacturer's precision claims and factor F (determined by degrees of freedom). Passing-Bablok regression and inter-rater agreement analysis were performed on 119 patient samples for Lupus S and 98 for Lupus C, for method comparison with the Sysmex CS-5100 analyzer (Sysmex Corporation, Kobe, Japan). Normalized ratios > 1.2 indicated lupus anticoagulant positivity.

Results: Lupus S met the precision criteria for low control, while Lupus C met them partially. For Lupus S, repeatability confidence intervals (CVs) were 1.2% and 1.8%; between-run, 1.5% and 3.0%; within-laboratory, 1.9% and 3.4%, for low and high controls, respectively. For Lupus C, repeatability CVs were 0.7% and 0.9%; between-run, 0.4% and 2.1%; within-laboratory, 0.7% and 2.2%, for low and high controls, respectively. Established RI were 24.1-32.6 s for Lupus S, 0.84-1.16 s for Lupus S ratio, 24.5-31.5 s for Lupus C, and 0.88-1.12 for Lupus C ratio, consistent with manufacturer specifications. Lupus S ratio had a constant difference ($N = 119$, $y = -0.34(-0.68 - (-0.12)) + 0.95(0.83 - 1.02)x$, $r = 0.983(0.973 - 0.990)$) with Sysmex CS-5100. Lupus C ratio showed both constant and proportional differences ($N = 98$, $y = -0.15(-0.21 - (-0.09)) + 1.14(1.09 - 1.20)x$, $r = 0.962(0.943 - 0.974)$). Agreement analysis of normalized LA-S/LA-C ratios showed excellent concordance, with Cohen's kappa at 0.90 (95%CI: 0.81 to 0.98).

Conclusion: The Lupus S and Lupus C dRVVT assays on the cobas t711 showed good analytical performance and suitable reference intervals. Despite minor constant and proportional differences, the normalized LA-S/LA-C ratios showed excellent concordance with the Sysmex CS-5100 analyzer, supporting their use in routine lupus anticoagulant testing.

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B-06 (Oral presentation)**Impact of normalization on the lupus anticoagulant diagnostic workflow**

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Introduction: Current guidelines for lupus anticoagulant (LA) testing recommend normalized ratios for result interpretation. This study evaluated the effect of normalization on the diagnostic workflow and interpretation of LA screen and screen/confirm tests using our laboratory's standard 3-step screen–confirm–mix algorithm.

Materials and methods: A paired analysis was performed on 193 samples from non-anticoagulated patients with a positive LA screening test. A previously verified manufacturer upper reference limit of 45.3 s was used to define non-normalized screen positivity. Samples were analyzed on the Sysmex CN-3000 coagulation analyzer using LA1 screening and LA2 confirmation dRVVT reagents, with Standard Human Plasma (SHP) used as a source of normal plasma dRVVT (all Siemens, Germany). Standard Human Plasma was tested with each new reagent lot. Non-normalized screen/confirm ratio (patient LA1 s/patient LA2 s), normalized screen ratio (patient LA1 s/SHP LA1s), and normalized screen/confirm ratio (patient LA1 s/patient LA2 s x SHP LA2 s/SHP LA1 s) were calculated. Cut-offs of 1.41 for non-normalized and 1.20 for normalized ratios were used for positive/negative classification. Classification differences were analyzed using the McNemar test in MedCalc statistical software (MedCalc, Ostend, Belgium), with $P < 0.05$ considered significant.

Results: Normalization of the screen test reclassified 25 results as negative (12.95%; 95% confidence interval (CI): 7.79 to 17.46%; $P < 0.001$), while normalization of the screen/confirm ratio reclassified 10 results as negative (5.18%; 95% CI: 2.05 to 8.31%; $P = 0.002$). Of the 10 reclassified screen/confirm ratios, 6 (60%) were borderline before (1.42-1.43) and after normalization (1.17-1.19).

Conclusion: Normalization of screen test results significantly affected classification and reduced the number of testing steps required for final interpretation. Although normalization of screen/confirm ratios also caused statistically significant reclassification, clinically relevant changes in diagnostic workflow and final interpretation occurred in only 5 (2.6%) cases, suggesting limited practical impact in our testing algorithm.

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B-07

Applicability of the harmonised prothrombin time reference interval: a multi-analyzer evaluation in healthy donors

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Introduction: Accurate reference intervals (RIs) for prothrombin time (PT) are crucial for ensuring consistent interpretation of the results. A harmonized cut-off value of $> 70\%$ for PT coagulometry is used in Croatian laboratories. We aimed to determine PT RIs for a commonly used reagent across the three leading coagulometers in healthy blood donors and evaluate their alignment with the harmonized national RI.

Materials and methods: Citrated plasma samples (N = 198) were obtained from healthy blood donors, aged 18-65 (median 49) years, under standardized preanalytical conditions. All samples were analyzed on three different systems: Siemens BCS XP (BCS), Sysmex CN3000 (CN), and CS2500 (CS), using Innovin (all Siemens, Marburg, Germany) for PT determination. The double-sided 95% RIs were calculated with 95% confidence intervals (CIs) for the lower and upper limits, as recommended by the CLSI Guidelines C28-A3, using the non-parametric method and were compared to the harmonised RI. Potential outliers were evaluated according to the Reed test. Statistical analyses were performed with MedCalc version 20.008 (MedCalc Software, Ostend Belgium).

Results: The obtained PT values across the platforms ranged from 75.8-140.6% for BCS, 71.0-130.1% for CN and 60.0-132.0% for CS, with median PT values of 111.9%, 100.6% and 97.3%, respectively. The PT RIs for each analyzer were as follows: BCS: 83.1% (95%CI: 77.6 to 87.0) - 136.7% (95%CI: 132.0 to 139.9); CN: 76.4% (95%CI: 73.6 to 80.8) - 121.1% (95%CI: 119.7 to 127.0), and CS: 67.9% (95%CI: 63.4 to 70.9) -126.0% (95%CI: 121.0 to 130.4), respectively.

Conclusions: Our results demonstrate that the lower limit of the reference interval differs across coagulation platforms. This highlights the importance of reviewing the currently used cut-off value to ensure accuracy and consistency. However, the clinical significance of this difference requires further evaluation.

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C Cardiovascular diseases

C-01

A review and analysis of N-terminal pro-B-natriuretic peptide ordering at Karlovac General Hospital

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Introduction: Due to its high negative predictive value N-terminal pro-B-natriuretic peptide (NT-proBNP) is an indispensable part of heart failure (HF) diagnostic procedure and it has prognostic significance in treatment of all cardiovascular diseases. Several recommendations are available for NT-proBNP minimum retesting interval (MRI). The aim of this study was to analyze NT-proBNP ordering at Karlovac General Hospital and recommended MRIs for NT-proBNP determination in order to rationalize laboratory costs.

Materials and methods: The number of requests ordered for NT-proBNP determination in the Department of Medical Biochemistry Laboratory, Karlovac General Hospital in a six-year period was evaluated in a retrospective analysis of data from the Laboratory Information System, and an analysis of ordered requests according to available MRIs for NT-proBNP was performed.

Results: Since 2019, the number of requests for NT-proBNP determination has been continuously increasing, and the increase in the number of requests in 2024 compared to 2019 is 575.5%. The largest part of requests for NT-proBNP determination pertains to outpatient requests (87.1%), followed by requests from departments without administrative restrictions (9.4%), and requests from departments with administrative restrictions (9.2%). In the three-year period, 19.2% of the total number of requests for NT-proBNP determination were repeated, of which 27.8% were repeated within 3 months, 29.8% within 6 months, and 42.3% within a year. The highest number of repeated requests, 85.5%, was from outpatient clinics.

Conclusion: The analysis of the number of requests for NT-proBNP determination showed an exponential increase in the number of requests in the observed period, with an emphasis on the increase in outpatient requests whose justification is questionable. Also, non-compliance with the recommended MRIs was observed in 19.2% of requests for NT-proBNP determination. The introduction of educational measures and/or MRI is expected to save €7,091.

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C-02

Comparison of calculated LDL cholesterol between Beckman Coulter and Siemens analyzers

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Introduction: Since 2025, the national screening program for familial hypercholesterolemia in Croatia has introduced the determination of calculated LDL cholesterol (LDL-C) in preschool children. In our previous study, we highlighted variability in total cholesterol results across analytical platforms, particularly between Beckman Coulter and Siemens analyzers. The aim of this study was to compare calculated LDL-C results derived from total cholesterol, HDL cholesterol, and triglyceride measurements obtained on Beckman Coulter and Siemens analyzers.

Materials and methods: The comparison was conducted using 40 serum samples from preschool children. Analyses were performed on the same day on the Atellica CH 930 (Siemens Healthcare Diagnostics, NY, USA) and DxC 700 AU (Beckman Coulter, Brea, USA) analyzers according to the manufacturers' specifications and using original reagents. LDL-C was calculated using the Friedewald equation. Method comparison was performed using Bland-Altman analysis and Passing-Bablok regression (MedCalc Software, Ostend, Belgium). A clinically significant difference was assessed by comparing the mean difference with the desirable bias criterion (6.35%) derived from within- and between-subject biological variation data from the EFLM Biological Variation Database.

Results: Passing-Bablok regression analysis showed no significant proportional difference between the methods (slope 1.00, 95% confidence interval (CI) 1.00 to 1.09), while a significant constant difference was observed (intercept 0.30, 95% CI 0.02 to 0.30). Bland-Altman analysis demonstrated a significant positive mean difference of 9.6% (95% CI 8.2% to 11.0%), indicating higher LDL-C values on the Beckman Coulter analyzer, with limits of agreement from 0.9% to 18.2%. The observed mean difference (9.6%) exceeded the desirable bias criterion (6.35%).

Conclusion: A consistent positive difference was observed, with higher LDL-C values on the Beckman Coulter analyzer, in line with previous observations for total cholesterol. The magnitude of this difference exceeded the selected clinical criterion and may influence the identification of children for further evaluation in familial hypercholesterolemia screening.

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C-03

Evaluation of the C-reactive protein to HDL cholesterol ratio as a marker of cardiovascular and renal risk among type 2 diabetic patients

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Introduction: Type 2 diabetes (T2D) is frequently associated with chronic low-grade inflammation, accelerated atherosclerosis, and progressive renal dysfunction. This study evaluated the association between the C-reactive protein (CRP)/HDL ratio, a composite marker of inflammation and anti-atherogenic capacity, and the presence of coronary artery disease (CAD) as well as the stages of chronic kidney disease (CKD) in patients with T2D.

Materials and methods: A total of 148 individuals with type 2 diabetes (T2D) were enrolled in this cross-sectional study, comprising 56 patients without (CAD) and 92 patients with CAD confirmed by angiography, along with 44 age-matched healthy controls. Renal function and chronic kidney disease (CKD) staging were determined using estimated glomerular filtration rate (eGFR) calculated through the CKD-EPI equation. Clinical characteristics and a broad range of biochemical, inflammatory, lipid, and renal markers were evaluated, and the CRP/HDL ratio was derived for each participant. Statistical analysis included intergroup comparisons, Spearman correlation testing, and receiver operating characteristic (ROC) curve analysis.

Results: Type 2 diabetes patients, particularly those with CAD, had significantly higher CRP levels, CRP/HDL ratios, blood pressure, glucose, and lipid abnormalities, along with lower eGFR than controls ($P < 0.001$). Progressive CKD stages were associated with worsening renal function, higher CRP, poor glycemic control, and dyslipidemia. The CRP/HDL ratio correlated positively with age, BMI, glucose, glycated hemoglobin, total cholesterol, triacylglycerols, LDL-C, and blood pressure, and negatively with eGFR. ROC analysis showed good predictive performance for CAD (AUC 0.82; sensitivity 78%, specificity 75%) with a cut-off of 2.2.

Conclusion: The CRP/HDL ratio is significantly associated with cardiovascular and renal complications in T2D, with elevated values reflecting increased inflammation, dyslipidemia, and renal dysfunction, supporting its potential as a simple biomarker for early identification of high-risk patients.

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D Endocrinology and bone diseases

D-01

Establishing of reference intervals for monomeric prolactin on Abbott Alinity i analyzer

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Introduction: Exclusion of macroprolactin is essential to avoid potential misdiagnosis and mismanagement of patients. So far, as of Abbott platforms, reference intervals (RIs) have been published only for Architect analyzer. Therefore, we aimed to establish RIs for monomeric prolactin on Abbott Alinity i analyzer.

Materials and methods: The study included 120 residual serum samples from apparently healthy males (N = 60) and females (N = 60) that visited University Hospital Sveti Duh as a part of routine physical examination. The samples were centrifuged, the serum was aliquoted and stored at -20°C until analysis. A 300 μL of serum was mixed with an equal volume of 25% polyethylene glycol (PEG) solution and incubated for 10 min at room temperature. The suspension was centrifuged for 10 min at 10,000 rpm and prolactin was measured in the resulting supernatant. The concentrations of both, total prolactin and post-PEG monomeric prolactin were determined by chemiluminescent microparticle immunoassay on Abbott Alinity i analyzer. The concentration obtained after PEG precipitation was multiplied by 2 due to dilution. The prolactin recovery was obtained from the formula: concentration after PEG precipitation (mIU/L) $\times 2$ / concentration the native sample (mIU/L) $\times 100$. MedCalc program version 12.5.0.0. (MedCalc, Ostend, Belgium) was used to calculate RIs (robust method, CLSI Guidelines C28-A3).

Results: Average concentrations of total vs post PEG monomeric prolactin (mIU/L) were: 233.46 (± 70.92) vs 193.85 (± 59.38) in male and 323.24 (± 101.62) vs 272.40 (± 89.11) in female participants. The average post PEG prolactin recovery (%) was 83.18 (± 6.27) in males and 84.75 (± 9.87) in females. Calculated reference interval for monomeric prolactin for males was: 61.68 mIU/L (95% confidence interval (CI) 41.64 to 87.64) – 303.90 mIU/L (95% CI 278.56 to 330.67) and for females: 89.68 mIU/L (95% CI 61.79 to 121.11) – 450.02 mIU/L (95% CI 416.18 to 480.53).

Conclusion: This is the first report of reference intervals for monomeric prolactin after precipitation with PEG on Abbott Alinity i analyzer.

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D-02

Can fasting insulin be a marker of risk for type 2 diabetes?

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Introduction: Insulin resistance is a condition in which cells have reduced sensitivity to insulin, and the pancreas compensates by increasing insulin production, leading to the development of prediabetes and type 2 diabetes. The importance of early detection methods for insulin resistance is evident, as this is a reversible condition. The aim of this study was to highlight the importance of isolated fasting insulin measurement. By the time tests such as HbA1c, fasting glucose and oral glucose tolerance test indicate abnormalities, patients have already developed significant insulin resistance and pancreatic beta-cell dysfunction.

Materials and methods: The study was conducted from 10 March 2025 to 4 March 2026. A total of 269 patients (aged 8-80 years, 86% female) referred to General Hospital Zabok for fasting insulin testing were included. Glucose concentration was determined using an enzymatic hexokinase method on the BC DxC700 analyzer, while insulin concentration was measured using a chemiluminescent method on the Abbott Alinity i analyzer. The HOMA1 index was calculated using a formula integrated into the Laboratory Information System ($\text{HOMA-IR} = \text{fasting insulin in pmol/L} \times \text{fasting glucose in mmol/L} / 156.26$), while the HOMA2 index was calculated using a publicly available online calculator (<https://www.dtu.ox.ac.uk/homacalculator/download.php>). The cut-off values used to exclude insulin resistance were < 2 for HOMA1 and < 1.22 for HOMA2.

Results: Fasting insulin values below 53.5 pmol/L indicate that the pancreas does not need to overproduce insulin to maintain normal blood glucose levels ($\text{HOMA1} < 2$, $\text{HOMA2} < 1.22$). Values between 53.5 and 67 pmol/L place patients in the so-called "gray zone", referring to borderline test results that are neither clearly positive nor clearly negative ($\text{HOMA1} 1.9-2.3$, $\text{HOMA2} 1.07-1.22$). Fasting insulin values above 67 pmol/L were observed in 154 patients (57%) and indicated insulin resistance according to the HOMA index ($\text{HOMA1} \geq 2$, $\text{HOMA2} \geq 1.22$).

Conclusion: The obtained data show that isolated fasting insulin measurement represents a valuable parameter that may reduce the number of undiagnosed individuals, especially those with insulin resistance, may influence patient treatment outcomes and ultimately impact healthcare costs.

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D-03

Immunometabolic signature of gestational diabetes mellitus: lymphocyte ratios and genetic risk factors

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Introduction: Gestational diabetes mellitus (GDM) is characterized by insulin resistance and chronic inflammation. This study investigated maternal immune shifts by evaluating NKT/NK, B1/B2, and T-cell subset ratios in women with GDM compared to healthy controls. Additionally, we quantified systemic levels of key cytokines (IL-6, TNF- α , IL-10, adiponectin) and their genetic variants, correlating them with biochemical and hematological parameters.

Materials and methods: The cross-sectional study included 162 pregnant women in their third trimester, categorized into four groups based on GDM and additional complications. Venous blood workup included: complete blood counts on Advia 2120i (Siemens); glucose, lipids, CRP, immunoglobulins, insulin, C-peptide, HbA1c, and ferritin on cobas pro (Roche); cytokines on Immulite 2000 (Siemens), adiponectin (ELISA, BioVendor R&D,) and lymphocyte immunophenotyping via BD FACSLyric (Becton Dickinson) flow cytometry. Genotyping (rs266729, rs1800629, rs1800896, rs1800796) was performed using the polymerase chain reaction with DNA quantification on Qubit (Thermo Fisher Scientific). Normality was tested via Shapiro-Wilk. Statistical analyses involved Chi-square, Mann-Whitney U, and Kruskal-Wallis tests. Correlations were assessed using Spearman's coefficient.

Results: No statistically significant differences in NKT/NK, B1/B2, or T-lymphocyte ratios were found between the GDM and control groups ($P > 0.05$). However, within the GDM population, a lower B1/B2 ratio was associated with elevated IL-6 levels ($r = 0.421$, $P < 0.01$) and demonstrated a significant negative correlation with adiponectin ($r = -0.388$, $P = 0.01$). Genetic analysis identified the CG genotype of the IL-6 gene (rs1800796) and the CC genotype of the ADIPOQ gene (rs266729) as significant risk factors for GDM development ($P < 0.05$). Notably, no significant associations were observed between these genotypes and their respective circulating serum cytokine concentrations ($P > 0.05$).

Conclusion: The CG genotype of IL-6 (rs1800796) and the CC genotype of ADIPOQ (rs266729) are associated with increased GDM risk. The significant relationship between the B1/B2-lymphocyte ratio and adiponectin in GDM patients suggests a complex interplay between humoral immunity and metabolic regulation.

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D-04

Short-term changes in thyroid function tests during fasting: a 2-hour follow-up study

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Introduction: Thyroid function tests may exhibit short-term variability influenced by physiological conditions. This study aimed to investigate whether significant differences exist in thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) concentrations between baseline morning status and two hours later, both in fasting state.

Materials and methods: This study included 31 healthy participants, with fasting serum samples collected at baseline (8:00 a.m., T0h) and 2 hours later (10:00 a.m., T2h). Serum TSH, FT4 and FT3 concentrations were measured by Roche electrochemiluminescent immunoassay. Data are presented as mean $\pm$ standard deviation for normally distributed variables and median (interquartile range) for non-normally distributed variables. Statistical analysis was performed to compare paired samples, with P-values < 0.05 considered significant.

Results: Thyroid stimulating hormone concentrations decreased significantly from a median of 1.83 mIU/L (1.37 to 2.58) at T0h to 1.37 mIU/L (0.90 to 1.86) at T2h ($P < 0.005$), corresponding to a median relative change of -25.6% (-29.9 to -20.0). Free triiodothyronine also showed statistically significant but small decrease from 5.2 ± 0.6 pmol/L at T0h to 5.1 ± 0.6 pmol/L at T2h ($P = 0.010$), with a median relative change of -1.3% (-2.8 to 0.2). In contrast, FT4 levels remained stable (15.3 ± 1.8 vs. 15.2 ± 1.7 pmol/L, $P = 0.365$), with no significant relative change (0.0% , -1.8 to 1.8).

Conclusion: In a fasting state, TSH and FT3 concentrations show statistically significant decreases within 2 hours, while FT4 remains unchanged. These findings suggest that variations occurring within a short period may influence the interpretation of thyroid function tests, particularly TSH, the first-line test in thyroid diagnostics. Standardization of sampling time should be considered in reliable clinical practice.

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D-05

Are the doctor's requirements for diagnosing thyroid disease in accordance with recommended guidelines?Tatjana Mikulin, Anamarija Đuras, Helena Kanižić

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Introduction: Laboratory diagnostics are readily available today, but not always in accordance with guidelines and rational laboratory practices. The aim of this research is to show whether the laboratory diagnosis of thyroid disease and determination of thyroid peroxidase antibody (anti-TPO) and thyroglobulin antibody (anti-Tg) is in accordance with the recommended guidelines. Questions were: How many anti-TG and anti-TPO tests were analyzed? How many results were within the reference interval? How many patients had TSH, FT3, FT4, anti-Tg, anti-TPO determinations on the same referral with results within reference interval (RI)?

Materials and methods: Data analysis was conducted on the results of patients referred to Medical Biochemistry Laboratory (MBL), General Hospital Varaždin in 2025. Data were extracted from the laboratory information system. Concentration of anti-Tg and anti-TPO were measured on an Abbott-Alinity analyzer using the chemiluminescent microparticle immunoassay.

Results: A total of 6172 anti-Tg and 6459 anti-TPO samples were analyzed in 2025. 68% results of anti-Tg and 72% results of anti TPO were within the reference limits. Inappropriate repeat testing was observed in 442 patients for anti-TPO and 512 patients for anti-Tg. A total of 3345 patients had all thyroid markers (TSH, FT3, FT4, anti-Tg, anti-TPO) ordered simultaneously on the same referral, among these, 1549 patients had results within RI across all parameters, representing an inappropriate referral rate of at least 46% within this group.

Conclusion: National guidelines from several countries agree on the same protocol for ordering anti-TPO and anti-Tg analysis. Anti-TPO should be measured only in case of primary hypothyroidism, subclinical hypothyroidism (two elevated TSH results) and without retesting. Anti-Tg should be ordered primarily to identify autoimmune thyroid diseases and to monitor individuals for recurrence of differentiated thyroid cancer and alongside the protein thyroglobulin. From the results obtained, we can conclude that physicians who refer patients to MBL do not follow the recommended guidelines.

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D-06

Vitamin testing: the most popular laboratory test or a justified demand?

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Introduction: Vitamin testing has increased substantially in recent years. This study assessed whether testing for vitamin D, B12, and folic acid at General Hospital Varaždin is clinically justified. Justification was evaluated according to the proportion of results outside the manufacturer's reference interval (RI), the frequency of repeat testing, and current clinical guidelines.

Materials and methods: All vitamin D, vitamin B12, and folic acid test results performed at General Hospital Varaždin in 2025 were retrospectively reviewed using data from the laboratory information system. All patients with at least one vitamin test were included. Measurements were performed using chemiluminescence immunoassays on the Abbott Alinity platform. The proportions of results within and outside the RI were determined.

Results: In 2025, 20,926 vitamin D, 17,289 vitamin B12, and 16,432 folic acid tests were performed. Of all results, 36% of vitamin D, 89% of vitamin B12, and 90% of folic acid measurements were within the RI. Repeat testing was common, accounting for 3054 vitamin D, 2013 vitamin B12, and 1921 folic acid tests. Most repeat B12 and folic acid results remained within the RI, indicating limited diagnostic value.

Conclusion: Without clinical diagnoses, the appropriateness of initial B12 and folic acid testing cannot be fully assessed. However, repeat testing appears largely unjustified because most initial results were already within the RI (89% and 90%), and repeat testing was frequent. In contrast, vitamin D testing appears justified for initial assessment, with 64% of results outside the RI, predominantly low. These findings are consistent with NICE and Choosing Wisely recommendations, which discourage routine vitamin D testing in asymptomatic individuals and recommend a minimum six-month retest interval. For B12 and folate, our findings support BSH guidance recommending testing primarily in patients with macrocytic anemia or neurological symptoms. Repeat testing should be limited to clear clinical indications.

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D-07

Routine laboratory results as the basis for a justified medical decision on the treatment of subclinical hypothyroidism

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Introduction: Clinical importance and treatment of mild subclinical hypothyroidism (SCH) remain controversial, and therapy decisions are individual and made by a medical doctor. We hypothesized that patients with a mild form of SCH who were prescribed levothyroxine therapy had significantly higher initial concentrations of biochemical and endothelial markers associated with cardiovascular risk when compared to SCH patients who were not prescribed levothyroxine. The aim was to study the differences in the initial concentrations of biochemical and endothelial markers between these two groups.

Materials and methods: The study included 61 patients with mild SCH, and levothyroxine was prescribed to 29 of them. Thyroid-stimulating hormone (TSH), free thyroxine (FT4), anti-thyroid peroxidase (anti-TPO), triglycerides, total cholesterol, LDL cholesterol and non-HDL cholesterol were determined in serum. Endothelial dysfunction markers (endocan, asymmetric dimethylarginine (ADMA), and endothelin-1 (ET-1)) were measured by ELISA (R&D Systems, Elabscience and Abcam, respectively). Data were analyzed using the independent samples t-test or Mann-Whitney test, depending on normality. $P < 0.05$ was considered statistically significant.

Results: Initially, SCH patients who were prescribed levothyroxine had significantly higher TSH (7.60 ± 1.47 vs. 6.81 ± 1.16 mIU/L, $P = 0.022$), anti-TPO (129.4 (8.6-344.6) vs. 6.85 (5.0-132.5) kIU/L, $P = 0.020$), LDL cholesterol (3.9 ± 0.8 vs. 3.4 ± 0.9 mmol/L, $P = 0.034$) and non-HDL cholesterol (4.5 ± 1.0 vs. 3.9 ± 1.0 mmol/L, $P = 0.044$), and lower FT4 (13.0 (12.75-14.25) vs. 15.0 (13.0-17.0) pmol/L, $P = 0.010$) comparing to patients not receiving therapy. ADMA and endocan concentrations were similar in both groups (332.60 (162.80-624.18) vs. 239.80 (190.95-431.90) $\mu\text{g/L}$, $P = 0.603$, and 91.47 (61.88-200.03) vs. 133.70 (70.01-192.60) ng/L, $P = 0.588$, respectively), while measured ET-1 concentrations were very low and clinically not significant.

Conclusion: The medical decision to introduce levothyroxine therapy in selected patients with a mild SCH based on routine laboratory assessment of thyroid status and dyslipidemia proved to be justified, highlighting the importance of timely diagnosis and treatment of SCH for the possible reduction of cardiovascular risk.

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E Chronic diseases

E-01

Trends in CA 19-9 testing and elevated values in 2015 and 2025: a retrospective study from Bitola

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Introduction: Carbohydrate antigen 19-9 (CA19-9) represents a tumor marker that is primarily used for detecting gastrointestinal malignancies with higher specificity for pancreatic and biliary tract cancers. This study aimed to analyze the progress of: trends in CA 19-9 testing, elevated values, and age/sex-specific patterns over a ten-year period at the Clinical Hospital "Dr. Trifun Panovski" Bitola.

Materials and methods: A retrospective review of CA 19-9 tests performed in 2015 and 2025 was conducted with collected results being classified as normal or elevated using standard CA 19-9 reference values. Distributions by age and sex were also assessed along with changes in total testing and elevated cases over the decade were analyzed.

Results: Collected results from this study show the following: in 2015 a total of 881 tests were performed, of which 682 (70.82%) had normal values with 199 (29.18%) having elevated values; while in 2025 a total of 1,898 tests were performed of which 1,666 (86.08%) had normal values with 232 (13.92%) having elevated values. Total number of tests conducted between the two years has risen by 115% while the number of elevated cases rose only slightly despite the lower proportion of abnormal results. Sex difference showed only a slight predominance in women tested individuals with women to ratios for normal values in 2015 being 1.6:1 and 2025 being 1.8:1. With the ratios for elevated values in 2015 being 1.6:1 and 2025 being 1.1:1 showing an equalizing distribution. Age differences within the study showed that most common higher values for CA 19-9 were individuals over 50 years of age with highest concentrations being found in the 61-80 age group.

Conclusion: Marker CA 19-9 testing has expanded over the decade, reflecting increased preventive screening, early detection, and monitoring of at-risk populations with a decrease in the proportion of elevated results, but still showing the improved participation and detection of gastrointestinal malignancies with in the population. These findings support the need for continued use of CA 19-9 for age-specific screening, monitoring and treatment of hepatopancreatobiliary due to the importance of integrating biomarker results with clinical contexts to optimize patient treatment and recovery.

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E-02

Comparison of the CKD-EPI creatinine equations from 2009 and 2021 in patients with diabetes mellitus

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Introduction: In Croatia, the estimated glomerular filtration rate (eGFR) is calculated using the 2009 CKD-EPI creatinine equation for adult patients. In 2021, the National Kidney Foundation (NKF) and the American Society of Nephrology (ASN) proposed a race-free equation. We aimed to compare the CKD-EPI creatinine equations from 2009 and 2021 in patients with diabetes mellitus (DM) at risk for the development of chronic kidney disease (CKD).

Materials and methods: Creatinine in serum, urine albumin, and creatinine were measured on AU5800 analyzers (Beckman Coulter, Brea, USA) with dedicated reagents, in 70 patients with DM from the University Hospital Dubrava. The urine albumin and creatinine ratio (UACR) was calculated. Calculation of eGFR was done with equations from 2009 and the 2021 race-free equation. Comparison of eGFR equations was done with Passing and Bablok (PB) regression, Bland-Altman analysis (BA), and Mann-Whitney test ($P < 0.05$). Chronic kidney disease heat map staging was done with both eGFR equations and UACR, and compared with a frequency table and Chi-square test. Statistical analysis was done in MedCalc Statistical Software (version 14.8.1, Ostend, Belgium).

Results: There is no statistically significant difference between eGFR 2009: 93.6 (89.0-100.1), and eGFR 2021: 98.0 (93.5-103.0) mL/min/1.73 m² ($P = 0.204$). PB regression ($y = 6.10 (4.49 - 8.71) + 0.97 (0.94 - 0.99) x$) ($P < 0.01$) showed constant and proportional difference. Result of BA analysis (mean difference and 95% confidence interval) was -4.3(- 8.6 to 0.0)%. Chronic kidney disease staging for patients was different when using eGFR 2009, and eGFR 2021: G1 (60.0 vs. 71.4%), G2 (21.4 vs. 11.4%), G3a (8.6 vs. 11.4%), G3b (8.6 vs. 4.3%), and G4 (1.4 vs. 1.4%); $\chi^2 = 179.398$; DF = 16, $P < 0.001$, cont.coeff = 0.848, respectively.

Conclusion: Our study showed no statistically significant difference in eGFR when using the formula from 2009 or 2021. Staging patients according to the CKD heatmap, based on different eGFR equations, led to different risk classifications and potentially changes in monitoring frequencies for some of the patients with DM in our study.

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F Autoimmune diseases

F-01

Association of CSF KFLC index with evoked potentials and MRI in early relapsing–remitting multiple sclerosis

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Introduction: The 2024 McDonald criteria introduced the kappa free light chain (KFLC) index as an additional cerebrospinal fluid (CSF) biomarker of intrathecal inflammation, alongside evoked potentials (EPs), which demonstrate dysfunction of central nervous system pathways. The aim of this study was to evaluate the association of these biomarkers, reflecting inflammation and neurodegeneration, in early relapsing–remitting multiple sclerosis (RRMS).

Materials and methods: The analysis included 72 consecutive treatment-naïve people with newly diagnosed RRMS (pwMS) (48 females; mean age 36.9 ± 11.1 years; median expanded disability status scale (EDSS) 2.5). All pwMS underwent a standardised diagnostic work-up, including brain and cervical spinal cord magnetic resonance imaging (MRI), CSF analysis with KFLC index calculation, and multimodal EP testing (visual EP (VEP), brainstem auditory EP (BAEP), and median and tibial somatosensory EP (mSSEP, tSSEP)). Pathological EP findings were summarised using an EP score.

Results: A higher KFLC index correlated with shorter latencies of left BAER III, bilateral tSSEP P40, and mSSEP N20, as well as higher amplitudes of left BAER V, right tSSEP P40-N50, and left mSSEP P15-N20. There was no statistically significant correlation between KFLC index values and EP pathological burden score. No significant associations were observed between KFLC index and clinical or neuroradiological parameters in this cohort.

Conclusion: In newly diagnosed RRMS pwMS, higher intrathecal inflammatory activity was paradoxically associated with less neurophysiological pathway dysfunction, predominantly in SSEP modalities, whereas no association was observed between EP scores and KFLC index values. These findings suggest that the KFLC index and EP scores capture distinct aspects of early MS neuropathology, emphasising their complementary role in applying new diagnostic criteria and evaluating MS patients.

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F-02

The clinical significance of zinc transporter 8 autoantibodies in patients with low-titer glutamic acid decarboxylase autoantibodies

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Introduction: Autoantibodies to glutamic acid decarboxylase (GADA) are the most widely used markers for diagnosing autoimmune diabetes. However, low-positive GADA titers (5.1-15 kIU/L) can present a diagnostic challenge, as they may reflect either an early autoimmune process or nonspecific positivity, particularly in patients with type 2 diabetes. Zinc transporter 8 autoantibodies (ZnT8A), a more recently introduced marker of islet autoimmunity, can serve as an additional tool to improve diagnostic accuracy. This study aimed to determine whether ZnT8A can improve diagnostic clarity in individuals with low-positive GADA results.

Materials and methods: A retrospective analysis was conducted between December 2025 and April 2026 on 31 serum samples from patients referred for autoimmune diabetes evaluation who had low-positive GADA levels (5.1 - 15 kU/L; diagnostic cut-off $\leq$ 5.1 kIU/L). The control group included 15 patients with negative islet autoantibodies, specifically GADA, islet cell autoantibodies (ICA), and tyrosine phosphatase-like protein autoantibodies (IA-2A). Zinc transporter 8 autoantibodies (cut-off $\leq$ 10 kAU/L), as well as other autoimmune markers, were determined using dedicated chemiluminescence immunoassays (CLIA) on the Maglumi X3 analyzer (Snibe Co. Ltd., Shenzhen, China).

Results: In the target group, 29 of 31 patients (93.5%) were negative for ZnT8A, predominantly with values below the method's limit of quantification (< 1 kAU/L). Likewise, all patients in the control group (15/15) were ZnT8A-negative, showing a similar distribution of low or undetectable values.

Conclusion: Zinc transporter 8 autoantibodies were negative in the majority of patients in both the target and control groups, with no significant differences observed between them. These findings suggest that low-positive GADA titers are frequently associated with nonspecific positivity rather than an active islet autoimmune process. Consequently, routine ZnT8A testing in this specific patient population does not appear justified. Its use should be reserved for selected clinical cases where supplemental data are essential for a definitive diagnosis.

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F-03

Distribution of cryoglobulinemia types according to sex and associated clinical diagnoses: a retrospective studyTajana Tunjić, Josip Knežević

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Introduction: This study aimed to analyze the distribution of cryoglobulinemia according to sex, cryoglobulin type, and associated clinical diagnoses. We hypothesized that immunological type III is the most frequent form and is more commonly associated with autoimmune diseases, with higher prevalence among female patients.

Materials and methods: A total of 76 positive patients of different age groups and with various clinical diagnoses were included in this retrospective study. Data were collected in the period from 24 February 2025 to 24 March 2026. The median age of the patients was 58 years (range 52-69). Variables analyzed included sex, year of birth, underlying clinical diagnosis, and cryoglobulin type (type II and type III). Data were processed using descriptive statistical methods, and results were presented as absolute and relative frequencies.

Results: Out of 76 patients, 59 (77.6%) were female and 17 (22.4%) were male. Immunological type III cryoglobulinemia was the dominant form, observed in the majority of patients (81.6%), while type II was significantly less frequent (18.4%). In female patients, type III (84.7%) was clearly predominant compared to type II, while in males type III (70.6%) also prevailed, with a relatively slightly higher proportion of type II compared to females. The most common associated diagnoses were Sjögren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis. Type II cryoglobulinemia was more frequently associated with monoclonal gammopathies.

Conclusion: The results indicate that immunological type III is the most common form of cryoglobulinemia and is predominantly associated with female patients and autoimmune diseases. The study hypothesis was confirmed, demonstrating a clear female predominance and strong association with systemic autoimmune diseases.

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F-04

Neurofilament determination in patients with suspected multiple sclerosis

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Introduction: Neurofilament light chain (NfL) is a biomarker of neuroaxonal injury increasingly used in the evaluation of multiple sclerosis (MS) and other neurodegenerative diseases. Kappa free light chain index (KFLCI) reflects intrathecal immunoglobulin synthesis and has emerged as a valuable diagnostic marker in MS. Combining biomarkers of inflammation and neurodegeneration may improve diagnostic assessment and disease monitoring. Aim of the study was to evaluate the association between KFLCI and cerebrospinal fluid (CSF) NfL in patients with suspected multiple sclerosis and to assess the potential clinical utility of neurofilament determination in routine diagnostic workup.

Materials and methods: This retrospective study included patients undergoing diagnostic evaluation for suspected demyelinating or other neurological diseases (N = 23). Kappa free light chains and albumin were measured nephelometrically using the Atellica NEPH 630 system (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany), and KFLCI was calculated from kappa free light chain and albumin quotients. Neurofilament light chain concentrations were determined by chemiluminescent immunoassay on the DxI 9000 analyzer (Beckman Coulter, Tokyo, Japan). Pearson's correlation analysis between KFLCI and CSF NfL concentrations was performed with MedCalc (MedCalc version 14.8.1, Ostend, Belgium).

Results: We found a statistically significant moderate positive correlation found between KFLCI and CSF NfL concentrations ($r = 0.61$, $P = 0.004$). In the subgroup of patients diagnosed with MS, a significant moderate correlation was also observed ($r = 0.58$, $P = 0.03$).

Conclusion: In this study higher KFLCI values were associated with increased NfL concentrations, suggesting simultaneous intrathecal immune activation and neuroaxonal damage. The observed association between KFLCI and NfL concentrations suggests potential complementary diagnostic and prognostic value of these biomarkers in demyelinating diseases. Neurofilament determination may represent a useful biomarker of neuroaxonal injury in patients with suspected MS but further studies on larger patient cohorts are required to confirm these findings.

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F-05

Analytical verification of DFS70 autoantibody testing in routine clinical laboratory practice

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Introduction: The anti-cell (AC)-2 pattern is defined by a dense, heterogeneous speckled staining of the nucleoplasm in interphase cells and the metaphase chromosomal plate. Distinguishing the dense fine speckled (DFS) pattern can sometimes be challenging. The objective of this study was to conduct an analytical verification of the chemiluminescence immunoassay (CLIA) for the detection of DFS70 autoantibodies, performed prior to its integration into routine clinical diagnostics.

Materials and methods: Detection of DFS70 autoantibodies is performed using CLIA on the BIO-FLASH analyzer (Biokit, Spain). Both within-run and between-run precision were assessed in accordance with the CLSI EP15-A3 guideline, using commercially available control materials at two concentration levels, analyzed in quintuplicate over five consecutive days. Serum samples used during verification were surplus patient specimens submitted for antinuclear antibodies (ANA) testing. The decision to test for DFS70 autoantibodies was based on the ANA pattern. Samples exhibiting a clear AC-2 pattern, or AC-2 pattern in combination with other patterns, were selected for DFS70 testing. Samples displaying an AC-1 pattern either alone or in combination with other patterns were also included.

Results: The within-run precision for the control material at level 1 (L1) was 3.0%, and 3.8% for level 2 (L2). The between-run precision was 7.5% for L1 and 5.9% for L2. The manufacturer-reported within-run precision values were 6.4% for L1 and 3.4% for L2, while for between-run precision were 1.4% for L1 and 2.7% for L2. During the verification, 158 serum samples were tested for DFS70 autoantibodies; 14 samples (8.9%) were positive (> 20 CU) and 11 (7.0%) had a concentration between 3.2-20 CU. Additionally, 60 samples tested positive for other autoantibodies included in the Luminex panel.

Conclusion: The results of the precision analysis didn't fully meet the manufacturer's criteria, but every clear AC-2 sample was tested positive for DFS70 autoantibodies which is of great help in routine work.

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H Pediatric laboratory medicine

H-01

Change in total bilirubin measurement method: impact on phototherapy decisions in neonatal hyperbilirubinemia

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Introduction: Total bilirubin (TBIL) measurement is essential for making the decision about phototherapy in neonatal hyperbilirubinemia. Method-dependent differences may affect clinical classification if decision thresholds are not adjusted. This study aimed to assess the impact of a method change on the classification of neonates according to the phototherapy threshold.

Materials and methods: The study included 50 neonatal serum samples. Total bilirubin concentration was measured on two analyzers, Beckman Coulter DxC700AU (Beckman Coulter, Brea, USA) and Roche Cobas Pro c 503 (Roche Diagnostics, Mannheim, Germany). Both TBIL methods are accredited according to ISO 15189. Method comparison was performed using Passing-Bablok regression and Bland-Altman analysis with MedCalc Statistical Software (version 22.005, MedCalc Software Ltd, Ostend, Belgium). Clinical impact was assessed using a locally applied phototherapy threshold of 250 µmol/L by determining the number of reclassified neonates after the method change.

Results: Total bilirubin concentrations ranged from 56 to 292 µmol/L (Beckman Coulter) and from 47 to 254 µmol/L (Roche Cobas). Median (interquartile range) values were 170 (95-250) µmol/L and 142 (82-213) µmol/L, respectively. Passing-Bablok regression showed both constant and proportional differences between methods: $y = -6.27$ (95% confidence interval (CI): -10.59 to -2.11) + 0.90 (95% CI: 0.87 to 0.93)x. Bland-Altman analysis showed a mean bias of 14.4% (95% CI: 13.4 to 15.4), with limits of agreement ranging from 7.3% to 21.5%, indicating consistently lower results on the Roche Cobas analyzer. On Beckman Coulter, 13/50 (26.0%) neonates had TBIL concentrations above the phototherapy threshold, compared with 1/50 (2.0%) on Roche Cobas. Consequently, 12/13 (92.3%) neonates classified above the threshold were reclassified below the threshold after the method change.

Conclusion: The change in TBIL measurement method resulted in systematically lower concentrations, leading to substantial reclassification of neonates below the phototherapy threshold. Retaining an unadjusted threshold may result in under-recognition of neonates requiring phototherapy, highlighting the need for method-specific decision limits.

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H-02

Comparison of procalcitonin concentrations in venous and capillary blood in newborns

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Introduction: As a biomarker, procalcitonin (PCT) plays a key role in the diagnosis of serious infections, including sepsis and severe bacterial inflammation. In addition to bacterial inflammation, certain conditions can cause elevated results, as can be seen in newborns; PCT concentration in the blood might be high in the absence of infection.

Materials and methods: The study was conducted in 100 serum samples from newborns in the period from 1st November 2025 to 1st April 2026 at the Clinic of Pediatrics and the Department of Laboratory Diagnostics of the University Clinical Hospital (UCH) Mostar (BiH). The PCT concentration was measured by the immunoturbidimetric method on an automatic analyzer Alinity ci (Abbott), and serum samples for determining the PCT concentration in venous and capillary blood were obtained by centrifugation of whole blood without anticoagulants for 10 minutes at 3500 rpm. Statistical data processing was performed in the MedCalc program (version 20.027, Ostend, Belgium).

Results: Statistical processing of the data revealed an abnormal distribution of the data, and the median and interquartile range (IQR) of PCT concentration in venous blood was 0.245 (0.095-0.720) ng/mL, and in capillary blood 0.205 (0.085-0.505) ng/mL. Comparing venous and capillary blood samples using the Mann-Whitney test, it was determined that there was no statistically significant difference in PCT concentrations, $P = 0.521$. Bland-Altman analysis determined the mean difference, $D = 0.0$, (12.7%) and limits of agreement (LOA) $D \pm 1.96s - 1.1 - 1.2$. The limits of the mean difference of the confidence interval included zero.

Conclusion: This research showed that there is no statistically significant difference in PCT concentrations in venous and capillary blood, as well as no constant and proportional deviation. This enables less invasive, faster and simpler blood sampling in newborns for more frequent checking of PCT concentration and eventual regulation of therapy in newborns in intensive care.

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H-03

Biochemical and hematological markers of acute inflammation 24 hours after minor elective surgery in children

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Introduction: Minor elective surgery refers to low-risk and minimally invasive procedures. Still, minor surgery and anesthesia can provoke development of mild to moderate acute inflammation. To assess this, we aimed to evaluate changes in biochemical and hematological inflammatory markers measured before and 24 hours after minor surgery.

Materials and methods: The study included 30 healthy children (age range 6-10 years), admitted at Children's Hospital Zagreb (CHZ) for minor elective surgery (phimosis, undescended testicle, hypospadias, hernia, strabismus). Exclusion criteria were chronic inflammatory conditions or a history of malignancy. The whole blood and serum samples were collected at two time points: immediately before and 24 hours after minor surgery for laboratory testing: complete blood count (Sysmex XN-1000, Kobe, Japan), C-reactive protein (CRP), haptoglobin (Hp) (both on Beckman Coulter AU 680, Mishima, Japan), procalcitonin (PCT) and interleukin-6 (IL-6) (both on Roche Cobas e411, Tokyo, Japan) determined at central laboratory of CHZ. The Wilcoxon rank sum test with $P < 0.05$ as the significance level was used to analyze changes.

Results: Medians of CRP, PCT, IL-6, Hp, white blood cell (WBC) count and relative/absolute neutrophil (Neu) counts were significantly lower immediately before minor elective surgery (CRP = 0.4 mg/L; PCT = 0.03 ng/mL; IL-6 = 1.5 ng/L; Hp = 0.7 g/L; WBC = 7.5×10^9 /L; Neu = 53.2%/ 3.7×10^9 /L) compared to the values measured 24h after surgery (CRP = 5.4 mg/L; PCT = 0.06 ng/mL; IL-6 = 16.7 ng/L; Hp = 0.9 g/L; WBC = 8.5×10^9 /L; Neu 69.8%/ 5.9×10^9 /L), all P values < 0.05 . Medians of relative/absolute lymphocyte (Lym) counts were significantly higher before (Lym = 37.0%/ 2.7×10^9 /L) than 24h after minor surgery (Lym = 20.5%/ 1.8×10^9 /L), $P < 0.05$. Relative/absolute monocyte counts (Mono = 7.5%/ 0.6×10^9 /L and Mono = 7.1%/ 0.7×10^9 /L) and platelet (324×10^9 /L and 302×10^9 /L) counts did not significantly changed during 24h after minor surgery ($P > 0.05$).

Conclusion: Our study confirmed significantly different values of most biochemical and hematological inflammatory markers, indicating that mild acute inflammation was provoked after minor elective surgery in children.

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H-04

Comparison of turbidimetrically and nephelometrically measured total IgG with calculated from sum of IgG subclasses

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Introduction: Immunoglobulin G (IgG) plays a key role in humoral immunity. It's four subclasses (IgG1-IgG4) are different in structure and functionality. Determination of IgG subclasses is important in diagnosis of immunodeficiencies and autoimmune diseases. The aim of this study was to compare turbidimetric and nephelometric methods for total IgG (tIgG) measurement with calculated sum of IgG subclasses.

Materials and methods: In 50 children aged 1 to 18 years, serum tIgG was determined by two methods: immunoturbidimetry (AU680, Beckman Coulter) and nephelometry (BN ProSpec, Siemens). IgG1-IgG4 subclasses were measured individually by nephelometry (BN ProSpec, Siemens). Comparison of methods for tIgG measurement was performed by Passing-Bablok regression analysis. The agreement of each method for tIgG with the sum of IgG subclasses was assessed by Bland-Altman analysis.

Results: The average concentration of tIgG was 10.23 g/L (range 3.90 to 18.20) for turbidimetry and 10.24 g/L (range 3.89 to 21.20) for nephelometry. Passing-Bablok regression analysis between turbidimetry and nephelometry showed no statistically significant systematic or proportional difference ($y = 0.005$ (95% confidence interval (CI): - 0.740 to 0.733) + 1.011(95% CI: 0.937 to 1.100)x). There was a relative bias of - 9.7% (- 24.7% to 5.3%) when compared turbidimetrically determined tIgG and the sum of IgG subclasses. Comparison of nephelometrically determined tIgG and the sum of IgG subclasses showed a negative bias of - 9.9% (- 29.7% to 9.9%) as well.

Conclusion: This study showed excellent agreement between turbidimetric and nephelometric methods for total IgG determination. However, comparison of measured with calculated (sum of IgG subclasses) total IgG, showed a consistent negative bias, regardless of total IgG assay method used. These discrepancies may be related to methodological limitations in IgG subclasses determination and should be taken into consideration in clinical interpretation.

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H-05

The verification of literature reference intervals for automated immature granulocyte measurements in neonatal capillary samples

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Introduction: Automated quantitative immature granulocyte (IG) measurement has been confirmed as a fast and reliable method on the Sysmex XN-1000 hematology analyzer (HA). We aimed to evaluate the potential applicability of automated IG measurement in neonatal complete blood count (CBC) samples and to verify the literature reference interval (RI) provided by the Children's Hospital of Philadelphia.

Materials and methods: A total of 30 neonatal whole blood K₂EDTA capillary samples without any of the morphological flags from the analyzer were included. Neonatal ages spanned from 1 to 23 days. Complete blood count measurements were performed on Sysmex XN-1000 HA. Blood smears were prepared and stained using the May-Grünwald-Giemsa technique and differentiated by an experienced medical laboratory scientist.

Results: Most samples were from babies ≤ 3 days old ($N = 20$). The provided RI ($< 0.28 \times 10^9/L$ absolute IG, $< 1.7\%$ IG) was applicable in 15/20 samples. In 3/20 samples, both absolute IG and IG% were increased, and immature granulocytes were also matched in manual differential at similar values. In 2/20 samples, the automated IG count increased (2.5% and 3%), with no immature granulocytes in the manual differential. Five babies belonged to the second RI category of 4-14 days ($< 0.27 \times 10^9/L$ absolute IG, $< 1.9\%$ IG); all five samples retrieved automated IG measurements within the RI, and immature granulocytes in the manual differential were negative. The same was true for the third RI category for babies older than 14 days, with automated IG measurements within the proposed RI ($< 0.22 \times 10^9/L$ absolute IG, $< 1.3\%$ IG).

Conclusion: Automated IG measurement in neonatal CBC samples is fast and reliable, and the results are within the literature RIs. For samples with increased IG counts, manual differentiation is recommended.

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H-06

Functionality of immunoglobulin concentration measurement below the lower limit of the measuring range on the CobasTM c303 system

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Introduction: Accurate determination of low immunoglobulins A/G/M concentrations is important in the diagnosis of immune disorders in children, particularly selective IgA deficiency. The aim of this study was to verify the functionality of immunoglobulin measurement below the lower limit of measuring range (LLMR) on the Roche Cobas c303 system.

Materials and methods: For standard application on the Cobas c303 analyzer, the declared LLMR values are 3.00 g/L for IgG and 0.25 g/L for IgM, while the highly sensitive IgA application has an LLMR of 0.10 g/L. The analyzer includes an increased sample volume pipetting functionality for measurements at low analyte concentrations. In 2-month and 18-day old child, serum IgA and IgG levels were below LLMR and IgM was close to LLMR. Nine serial dilutions of primary sample were prepared in ratios from 1+1 to 1+9. Immunoglobulin A/G/M were measured using the increased sample volume mode. Measured concentrations for each dilution were compared with corresponding theoretical concentrations calculated from the accurately measured native sample concentration.

Results: The concentrations of IgG, IgM and IgA in native sample were 2.560, 0.393 and 0.0649 g/L, respectively. The mean measured concentrations in diluted samples using increased sample volume mode were 2.627 g/L (standard deviation (SD) = 0.047) for IgG, 0.348 g/L (SD = 0.012) for IgM and 0.0639 g/L (SD = 0.0049) for IgA. The average deviation between measured and theoretical concentrations in diluted samples was 2.63% for IgG, - 11.52% for IgM and - 1.60% for IgA. The lowest concentrations that could be reliably measured using the increased sample volume mode were 0.427 g/L for IgG and 0.0561 g/L for IgM.

Conclusion: The increased sample volume pipetting functionality on the Cobas c303 analyzer enables reliable measurement of immunoglobulin A/G/M concentrations below the declared LLMR. New lower reporting limits were confirmed at 0.4 g/L for IgG, 0.05 g/L for IgM and 0.04 g/L for IgA.

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I Laboratory toxicology

I-01 (Oral presentation)

Is measuring carbamazepine concentration sufficient for therapeutic drug monitoring in patients receiving carbamazepine therapy: a pilot study

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Introduction: Carbamazepine (CBZ) is metabolized into two main metabolites: the active and potentially toxic carbamazepine-10,11-epoxide (CBZ-EPO), and the inactive, non-toxic 10,11-dihydroxycarbamazepine (CBZ-DIOL). The ratio of metabolite to parent drug (CBZ-EPO/CBZ) may indicate an increased risk of neurotoxicity due to reduced degradation of the neurotoxic CBZ-EPO. The aim of this pilot study was to evaluate the clinical usefulness of measuring CBZ metabolites using liquid chromatography–tandem mass spectrometry (LC–MS/MS). We hypothesized that metabolite measurement provides additional value in therapeutic drug monitoring (TDM).

Materials and methods: Blood samples for trough concentration measurement were collected in the morning prior to the next dose, using tubes without clot activator and gel (Greiner Bio-One GmbH, Kremsmünster, Austria). Concentrations of CBZ, CBZ-EPO, and CBZ-DIOL were determined in serum using an LCMS-8050 analyzer (Shimadzu, Kyoto, Japan) with the commercial ClinMass Add-On Set for Antiepileptics (Recipe, Munich, Germany). Only patients with CBZ concentrations within the therapeutic range (17–50 µmol/L) were included in the study. The CBZ-EPO/CBZ ratio was calculated, with values < 0.2 considered normal, 0.2–0.3 borderline, and > 0.3 indicative of increased risk of neurotoxicity. Data are presented as median and interquartile range and were analyzed using MedCalc Statistical Software version 20.015 (MedCalc Software Ltd, Ostend, Belgium).

Results: A total of 27 patients met the inclusion criteria. Median (interquartile range) concentrations of CBZ, CBZ-EPO, and CBZ-DIOL were 30 (26–35) µmol/L, 5 (3–8) µmol/L, and 13 (5–19) µmol/L, respectively. Fifteen patients had a CBZ-EPO/CBZ ratio < 0.2 (normal), 8/27 had values between 0.2 and 0.3 (borderline), and 4/27 had ratios > 0.3 (increased).

Conclusion: Although all patients had CBZ concentrations within the therapeutic range, 12 showed potentially altered pharmacokinetics, of whom 4 had a significantly increased risk of neurotoxicity. Measurement of CBZ metabolites improves the clinical utility of CBZ TDM.

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I-02 (Oral presentation)

Indirect estimation of selenium reference intervals: comparison of CLSI C28-A3 and RefineR approachesNora Nikolac Gabaj^{1,2}, Matej Nekić¹, Marija Božović¹, Iva Tarnik¹¹Department of Clinical Chemistry, Sestre milosrdnice University Hospital Center, Zagreb, Croatia²Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Introduction: Selenium is an essential trace element involved in numerous physiological processes. Measurement of its concentration is increasingly used to assess both deficiency and toxicity. For in-house methods, reference intervals (RI) are often adopted from the literature. This study aimed to calculate indirect RIs using retrospective data by applying two approaches (CLSI C28-A3 and RefineR) and to compare them with the interval proposed by the Croatian Chamber of Medical Biochemists (CCMB).

Materials and methods: This retrospective study was conducted using results extracted from the laboratory information system (LIS) for the period from 11/2022 to 02/2026. Selenium concentration was determined by graphite furnace atomic absorption spectroscopy using a 240Z AA analyzer (Agilent Technologies, Santa Clara, USA). For the CLSI C28-A3 protocol, data normality was assessed by Kolmogorov–Smirnov test. Outliers were identified and excluded using the Tukey method. The influence of age and sex was evaluated by multiple linear regression. Statistical analysis was performed using MedCalc (MedCalc Software Ltd, Ostend, Belgium). Reference intervals were calculated as the 2.5th–97.5th percentiles. The RefineR method was applied without prior exclusion of outliers using the online RefLimR tool (available at: https://gocrunch.shinyapps.io/RefLimR_Derive_RI/; Accessed: March 24, 2026). CCMB RI was 0.64–1.52 µmol/L.

Results: A total of 1694 results were retrieved from the LIS. After exclusion of external quality assessment samples, 1640 results were further analyzed. Due to non-normal distribution ($P < 0.001$), logarithmic transformation was applied. Following outlier exclusion, 1578 results were used for RI calculation. Multiple regression analysis showed no statistically significant effect of age ($P = 0.940$) or sex ($P = 0.983$), indicating that stratification was not required. The calculated RI using the CLSI approach was 0.73–1.73 µmol/L, while the RefineR method yielded similar results (0.74–1.83 µmol/L).

Conclusion: Indirectly obtained reference intervals were comparable between methods and slightly higher than the CCMB interval, possibly reflecting increased selenium supplement intake.

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I-03

Tacrolimus blood concentration monitoring in the first year after kidney transplantationNafija Serdarević^{1,2}, Suzana Tihić-Kapidžić^{1,3}¹Department of Clinical Biochemistry, University Clinical Center, Sarajevo, Bosnia and Herzegovina²Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina³Pharmacy faculty, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Introduction: Tacrolimus is macrolide lactone immunosuppressive drug and it is a calcineurin inhibitor. The main action of tacrolimus in the human body is the inhibition of T-cell proliferation by binding to FK506-binding protein (FKBP). As tacrolimus shows significant inter-/intra-patient variation in its pharmacokinetics, narrow therapeutic index, and poor bioavailability (oral), therapy with tacrolimus is challenging. The therapeutic drug monitoring of tacrolimus is very important to prevent intoxication or rejection due to incorrect dosage and achieve the target range of 7-12 ng/ml. The aim of our study was determination of tacrolimus during the first year, starting one month after renal transplantation.

Materials and methods: Investigation included 163 blood samples that were collected from 11 transplant patients (5 men and 6 women), age range 29.3 +/- 5.4, in the period February 2025 till February 2026. The blood analyses were performed on the CMIA (Alinity analyzer; Abbott, USA). Statistical analysis was carried out using MedCalc and SPSS 20. The concentration of tacrolimus was measured once a month in all patients, in the morning before taking the first dose of the medication. In the case that we got high or low concentration, we repeated sampling the next day.

Results: The concentration of tacrolimus varied from 3.1 to 18.7 ng/ml. The recommended concentration (from 7 to 12 ng/ml) of tacrolimus was in 81.8% of patients considered for individual therapy. In women patients (66.6%), we got lower concentrations between 5.5 and 9.5 ng/ml in comparison with men patients. Comparing the median tacrolimus concentration between the male and female patients, there was a statistically significant difference, $P < 0.05$.

Conclusion: Regular monitoring of tacrolimus blood concentration is necessary after kidney transplantation to achieve the optimal dose for each patient.

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I-04

Can oxcarbazepine be excluded in therapeutic drug monitoring? A retrospective LC/MS risk assessment study

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Introduction: Oxcarbazepine is a prodrug with minimal antiepileptic activity and is rapidly metabolized to its active metabolite, 10-hydroxycarbazepine (MHD). The parent compound has a very short detection window and is preanalytically unstable. For therapeutic drug monitoring (TDM) purposes, the reported concentration represents the sum of oxcarbazepine and MHD concentrations. We hypothesized that direct measurement of oxcarbazepine may be unnecessary, and monitoring of MHD alone would be sufficient for TDM. The aim of the study was to evaluate the contribution of oxcarbazepine to the reported total concentration and to assess whether excluding its measurement would have any clinically relevant impact.

Materials and methods: For this retrospective study trough serum concentrations of oxcarbazepine and MHD were measured in steady-state samples using liquid chromatography–mass spectrometry LCMS-8050 (Shimadzu, Japan) with the ClinMass Add-On Set for Antiepileptics (Recipe, Munich, Germany). Following quantification of both oxcarbazepine and MHD, their summed concentrations were calculated, and the relative contribution of oxcarbazepine to the total sum was determined using equation as follows: oxcarbazepine proportion = $c(\text{oxcarbazepine}) / c(\text{oxcarbazepine} + \text{MHD}) \times 100$. The reported therapeutic range for summed concentration was 40–138 $\mu\text{mol/L}$. A retrospective risk assessment was performed to evaluate the clinical impact of excluding oxcarbazepine from routine measurement based on its contribution to the total concentration. Data analysis was performed in Excel, and results are presented as median and interquartile range.

Results: A total of 53 patient samples with paired measurements of oxcarbazepine and MHD were included in the analysis. The median proportion (interquartile range) of oxcarbazepine in the total concentration was 2% (1–2%). In 27 of 53 samples, oxcarbazepine concentrations were below 1 $\mu\text{mol/L}$.

Conclusion: Oxcarbazepine contributes only minimally to the total measured concentration and does not affect the interpretation of therapeutic drug monitoring. Therefore, its measurement could be excluded, with the reported concentration effectively reflecting MHD.

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I-05 (Oral presentation)**Area under the curve: a valuable indicator of mycophenolic acid dosing success**

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Introduction: The clinical pharmacokinetics of mycophenolic acid (MPA) are characterized by inter- and intra-individual variability. Given the poor correlation of trough MPA concentrations with efficacy and safety, therapeutic drug monitoring is preferably based on area under the curve (AUC) determination, which is widely regarded as the best measure of overall drug exposure to MPA. Since determination of the full AUC is difficult to implement in clinical practice, simpler approaches are needed to reliably estimate MPA-AUC values. The aim of this study was to analyze trough MPA (C₀) concentration and AUC value calculated from limited sampling and compare this value with the proposed therapeutic window.

Materials and methods: To assess abbreviated pharmacokinetic profiles, plasma samples from a cohort of 67 patients were collected at 0 min (C₀), 60 min (C₁), and 120 min (C₂) post-dose. Plasma MPA concentrations were analyzed using gradient reverse-phase high-performance liquid chromatography with diode-array detection. The MPA-AUC values were calculated using the formula: $AUC = 8.7 + 4.63 \times C_0 + 1.90 \times C_1 + 1.52 \times C_2$.

Results: Median (interquartile range, IQR) MPA concentrations were as follows: C₀ = 2.3 (1.3-3.3) mg/L, C₁ = 8.5 (4.9-14.0) mg/L, and C₂ = 5.8 (3.7-9.5) mg/L. Peak MPA concentrations occurred at C₁ in 78% of patients, at C₂ in 19%, and at C₀ in 3%. The median (IQR) MPA-AUC value was 43.7 (35.6-65.1) mg·h/L. MPA-AUC values in 48% of patients were within the therapeutic range of 30-60 mg·h/L, whereas 16% were below and 36% were above this range.

Conclusion: The results demonstrated variability in the pharmacokinetic profiles of MPA among patients, with peak concentrations occurring at different time points. Although median AUC values were within the proposed therapeutic window, only half of the patients achieved the target range, confirming high pharmacokinetic variability and the need for individualized dose adjustment.

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J Molecular diagnostics

J-01

Pharmacogenetics of brivaracetam: impact of CYP2C9 and CYP2C19 variants on therapeutic drug monitoring in real-life data

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Introduction: Brivaracetam is an antiepileptic drug primarily metabolized *via* hydrolysis, while minor metabolic pathways involve CYP2C19 and CYP2C9, whose genetic variability may influence brivaracetam pharmacokinetic. The aim of this study was to evaluate the relationship between CYP2C9 and CYP2C19 genotypes/phenotypes and serum brivaracetam concentrations in a real-life clinical setting.

Materials and methods: This retrospective study analysed data from patients with epilepsy treated with brivaracetam. Genotyping for CYP2C9*2, CYP2C9*3, CYP2C19*2 and CYP2C19*17 was performed using TaqMan Real-Time PCR, while serum steady-state trough brivaracetam concentrations were measured by HPLC-DAD, with all methods validated in a clinical laboratory. Brivaracetam concentrations, dose-adjusted concentration (D/C) ratios, clinical data, drug interactions, and genotype/phenotype data were analysed in univariate statistical analysis.

Results: We analysed data from 50 patients (29 males, 21 females) aged 6-76 years (mean = 31), receiving brivaracetam doses 50-200 mg/day, brivaracetam concentrations ranged 0-21.6 µmol/L. CYP2C19 poor metabolizers (*2/*2, N = 2) exhibited 55% higher D/C ratios compared to normal metabolizers (*1/*1, N = 20; P = 0.02); intermediate metabolizers (*1/*2/*17, N = 11) showed 20% higher exposure (P = 0.04); ultra-rapid metabolizers (*17/*17, N = 3) had subtherapeutic concentrations (median: 2.8 µmol/L at 200 mg; P = 0.03). CYP2C9 heterozygous carriers (*1/*2, *1/*3, N = 18) demonstrated 22% higher D/C ratios compared to wild-type (*1/*1, N = 32; P = 0.12), reaching significance in patients on CYP2C9 inhibitors (valproate) (Δ = 38%, P = 0.01). Enzyme inducers (carbamazepine, phenobarbital) reduced brivaracetam concentrations by 40-60% in NMs (P < 0.01).

Conclusion: This small real-life study showed that genetic variations in CYP2C19, and less in CYP2C9, influence brivaracetam concentrations, and their interaction with inducers/inhibitors necessitates vigilance. These findings warrant further investigation in larger cohorts.

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J-02

Analytical and clinical verification of digital PCR for c-KIT mutations in cfDNA across breast cancer subtypes and stagesAndro Brzović¹, Sandra Šupraha Goreta¹, Marko Žarak^{2,3}¹Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia²University Department of Laboratory Diagnostics, Dubrava University Hospital, Zagreb, Croatia³Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Introduction: Breast cancer is a heterogeneous disease characterized by diverse molecular alterations and inter- and intra-tumor variability across clinical stages and molecular subtypes. Although c-KIT mutations are well established in other malignancies, particularly hematological neoplasms, and are involved in signaling pathways regulating cell proliferation, differentiation, and survival, their role in breast cancer remained insufficiently explored. This study aimed to analytically and clinically verify the application of digital polymerase chain reaction (dPCR) for detecting the c-KIT D816V mutation using genomic DNA (gDNA) and circulating cell-free DNA (cfDNA) obtained from breast cancer patients.

Materials and methods: A total of 31 female patients with breast cancer were included in the study. Peripheral blood and plasma samples were collected for gDNA and cfDNA isolation. DNA quality and quantity were assessed prior to analysis. Digital PCR assay (NioE, Stilla Technologies, France) targeting c-KIT D816V mutation was verified by evaluating repeatability, intermediate precision, limit of detection (LoD) and concordance with allele-specific PCR (ASO-PCR).

Results: The LoD was 0.156 ng/μL, confirming high analytical sensitivity. Repeatability (CVr) ranged from 7.90% to 54.31%, with the highest variability seen at low mutant-allele concentrations (mean 1.07 copies/μL; Sr = 0.58). Intermediate precision (CVb) ranged from 2.53% to 19.61%. Comparison with ASO-PCR showed poor agreement (Cohen's kappa = 0.200), likely reflecting reduced specificity and false-positive findings associated with ASO-PCR. Furthermore, no c-KIT D816V mutation was detected in any of the analyzed gDNA or cfDNA samples.

Conclusion: Digital PCR represents a highly sensitive and robust method for mutation detection in liquid biopsy samples, with potential for integration into routine molecular diagnostics. However, further assay optimization, standardization, and validation are required. The absence of c-KIT mutations in this cohort suggests that they are unlikely to represent clinically relevant biomarkers in breast cancer.

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J-03

Impact of hemolysis, icterus and lipemia on PCR melting curve analysis using a LightCycler 480-based workflow

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Introduction: Hemolysis, icterus, and lipemia (HIL) are common preanalytical interferences in clinical laboratory practice. While their impact on biochemical assays is well established, their effect on molecular diagnostics, particularly real-time PCR and melting curve analysis, remains insufficiently characterized. This study evaluated the effects of HIL on PCR melting curve analysis using a LightCycler 480-based workflow.

Materials and methods: For each interference type, eight K₃EDTA-whole blood samples were used, with each sample divided into a control and two interference-level aliquots. Hemolysis was induced mechanically by repeated aspiration through a 21G needle to achieve levels of 1-4 (HB) and 4.1-15 g/L (HC) of free hemoglobin. Icterus was simulated by adding a commercial direct bilirubin standard to obtain bilirubin concentrations of 280-600 (IB) and 601-1032 µmol/L (IC). Lipemia was induced by adding 20%-Intralipid to achieve triglyceride concentrations of 4-12 (LB) and 12.1-23 g/L (LC). DNA was extracted using High Pure PCR Template Preparation Kit (Roche). Melting curve analysis for PAI-1 detection was performed using LightMix Kit (TIB MOLBIOL) on LightCycler 480 II (Roche). Melting temperature (T_m) and PCR success rate were recorded. Differences across groups were assessed using Friedman test (MedCalc v20.008).

Results: Friedman test revealed a statistically significant effect of icterus on T_{m1} (P = 0.008). *Post-hoc* multiple comparisons showed differences between control and both icteric levels (IB and IC). However, mean ΔT_{m1} for both icteric levels (0.31°C and 0.24°C, respectively) remained within the manufacturer's declared limit of 0.5°C. No statistically significant effect of hemolysis or lipemia on T_m was observed. Amplification was successful in all samples, with complete concordance of genotyping results across all conditions.

Conclusion: Hemolysis and lipemia had no effect on T_m values, while icterus caused statistically significant but analytically insignificant T_{m1} shifts. All samples amplified successfully, with complete genotyping concordance, indicating robustness of this specific extraction-PCR workflow to HIL interference.

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J-04

Comparison of two microvolume spectrophotometers for DNA concentration measurement and purity ratio assessment

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Introduction: Microvolume UV spectrophotometers are widely used for DNA quality control, yet comparability across platforms remains uncertain. This study evaluated comparability between the DeNovix DS-11 FX+ (DeNovix Inc.) and NanoPhotometer N60 (Implen) for DNA concentration measurement and purity ratio assessment.

Materials and methods: DNA was isolated from 42 EDTA-whole blood samples using the High Pure PCR Template Preparation Kit (Roche). All isolates were measured in duplicate, and mean values were used for statistical analysis. DNA concentration was compared in all samples, whereas A260/A280 and A260/A230 ratios were evaluated only in 28 samples with DNA concentration > 20 ng/μL, as absorbance-based purity assessment is considered reliable at this concentration. Method comparison included Bland-Altman analysis and Passing-Bablok regression, while Spearman correlation assessed association (MedCalc v20.008). Categorical agreement for purity ratios was assessed as percentage agreement using predefined ranges based on commonly used literature criteria (A260/A280: 1.7-2.0; A260/A230: 1.8-2.2).

Results: A very strong correlation was observed for DNA concentration ($\rho = 0.984$), with a mean bias of - 0.92%. However, Passing-Bablok identified both constant (intercept - 2.21; 95% CI - 3.52 to - 1.21) and proportional difference (slope 1.08; 95% CI 1.05-1.14), indicating that the two instruments were not fully interchangeable across the measuring range. For A260/A280 and A260/A230, Bland-Altman and Passing-Bablok showed no significant systematic differences. Categorical agreement was higher for A260/A280 than A260/A230 (92.9% vs 82.1%), indicating good concordance for A260/A280 but lower for A260/A230.

Conclusion: The two spectrophotometers demonstrated close agreement for DNA concentration, although systematic constant and proportional differences were detected. Purity ratio classification was more consistent for A260/A280 than A260/A230, the latter showing only moderate categorical concordance. These findings suggest that DNA concentration results from the two instruments are broadly comparable but not strictly interchangeable across the full measuring range. Purity ratios, particularly A260/A230, should be interpreted cautiously when fixed decision thresholds are applied.

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J-05

Capillary vs. polyacrylamide gel electrophoresis after multiplex PCR-based clonality testingŽeljka Tkalcic Švabek¹, Mirjana Mariana Kardum Paro¹, Delfa Radić Krišto², Slavko Gašparov³¹Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia²Internal Medicine Clinic, Merkur University Hospital, Zagreb, Croatia³Clinical Department for Pathology and Cytology, Merkur University Hospital, Zagreb, Croatia

Introduction: Molecular analysis of B- and T-lymphocyte clonality is important in the diagnosis of lymphoproliferative disorders (LPDs). The detection of V-J gene rearrangements is commonly performed with multiplex polymerase chain reaction (PCR) according to EuroClonality/BIOMED-2 guidelines, followed by fragment analysis using capillary electrophoresis (CE). The aim of this study was to validate a multiplex PCR clonality testing method using CE with comparison to results previously obtained by polyacrylamide gel electrophoresis (PAGE), and assess its suitability for routine diagnosis of LPDs.

Material and methods: A total of 12 DNA samples from peripheral blood, bone marrow, lymph nodes, formalin-fixed tissue and EQA material were analyzed for each gene rearrangement, including immunoglobulin heavy (IGH) and kappa light chains (IGK), T-cell receptor beta (TRB) and gamma (TRG), using multiplex PCR based on EuroClonality/BIOMED-2 guidelines with commercially available assays (Gene Clonality Assay, Invivoscribe, USA). Amplification products were analyzed using PAGE or fragment analysis by CE and results were interpreted as clonal or polyclonal based on band position or fragment size distribution. Reproducibility was assessed in 5 clonal and 5 polyclonal samples within a single run. Agreement between methods was evaluated using Cohen's kappa coefficient (MedCalc version 22.005, MedCalc Software Ltd, Ostend, Belgium).

Results: A concordance of 100% (12/12) was observed for each gene rearrangement (IGH, IGK, TRB and TRG). All Cohen's kappa coefficients were 1.0. In repeated measurements, consistent results were observed for clonal (5/5; 100%) and polyclonal (5/5; 100%) samples. No differences in the interpretation of clonality between detection methods were observed.

Conclusion: Clonality results of B- and T-lymphocytes assessed by PAGE, accredited according to EN ISO 15189, were identical to those obtained by CE, with kappa analysis indicating complete agreement between the methods. Multiplex PCR based on EuroClonality/BIOMED-2 guidelines with fragment analysis by CE meets analytical performance criteria and is suitable for routine LPDs diagnostic.

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J-06

Comparison of DeNovix dsDNA broad range assay and genomic DNA ScreenTape assay for gDNA quantification

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Introduction: Accurate quantification of genomic DNA (gDNA) is essential for the optimal performance of molecular diagnostic methods such as quantitative polymerase chain reaction (qPCR) or sequencing, and fluorometry is considered the most reliable routine approach for gDNA quantification due to its high specificity. The DeNovix dsDNA Broad Range assay enables routine fluorometric quantification of gDNA, whereas the Genomic DNA ScreenTape assay combines electrophoretic separation and fluorescent detection, allowing simultaneous assessment of DNA integrity and concentration. This study aimed to compare gDNA concentrations obtained using these two assays.

Materials and methods: Genomic DNA was isolated from 33 K₃EDTA whole blood samples using the High Pure PCR Template Preparation Kit (Roche). Genomic DNA concentration was measured in duplicate using the DeNovix dsDNA Broad Range assay (DeNovix fluorometer) and the Genomic DNAScreenTape assay (Agilent 4200 TapeStation). For each sample, duplicate measurements were averaged prior to statistical analysis. The association between the two assays was assessed using Spearman correlation coefficient, while method comparison was performed using Bland-Altman (BA) and Passing-Bablok regression (PB) analyses (MedCalc v20.008, Ostend, Belgium).

Results: A strong positive correlation was observed between the two assays ($p = 0.94$; 95% confidence interval (CI): 0.89-0.97). Mean DNA concentrations (ng/ μ L) were 30.1 (95% CI: 25.5-34.7) for DeNovix and 24.5 (95% CI: 19.1-30.0) for ScreenTape, with an average bias of - 20%. The BA demonstrated both constant and proportional differences between the assays (5.58 (95%CI: 3.20-7.96) and 23.97% (95%CI: 17.92-30.01), respectively) while PB indicated a proportional difference, with a slope of 0.77 (95% CI: 0.66-0.94).

Conclusion: Although a strong correlation between the two assays was observed, BA and PB demonstrated constant and proportional bias. The Genomic DNA ScreenTape assay yielded lower gDNA concentrations than the DeNovix dsDNA Broad Range assay in this sample set. These differences should be considered when the two methods are used interchangeably, particularly in applications requiring precise DNA input.

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J-07

Structural DNA degradation during long-term refrigerated storage has limited impact on PCR-based genotyping in a single assay

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Introduction: According to CLSI MM13 guidelines, purified DNA should be stored at + 2 to + 8°C for up to one year, while long-term storage is recommended at - 20°C or lower. However, DNA isolates are often stored longer under refrigeration. We evaluated changes in DNA quality parameters and PCR genotyping performance after prolonged refrigerated storage.

Materials and methods: DNA isolates (N = 223) from EDTA-blood samples were extracted using MagMAX DNA Multi-Sample Ultra 2.0 Kit on KingFisher Flex (Thermo Fisher Scientific) and stored at + 2°C to + 8°C for up to 55 months. Paired measurements were performed at 18 and 55 months. DNA concentration and purity (A260/A280, A260/A230) were assessed using DeNovix DS-11 FX+ (DeNovix). DNA integrity was evaluated by DNA Integrity Number (DIN) using Genomic DNA ScreenTape on 4200 TapeStation System (Agilent Technologies). Isolates were measured in duplicate; mean values were used. PCR performance and genotype concordance were assessed using LightMix ACE I/D assay (TIB MOLBIOL) on LightCycler 480 II (Roche). Statistical analysis included Wilcoxon signed-rank and McNemar exact tests (MedCalc v20.008, Ostend, Belgium).

Results: DNA integrity decreased over time (median DIN 9.6 vs 7.5; $P < 0.001$), with samples having DIN < 7 increasing from 7.6% to 38.1% ($P < 0.001$). A260/A230 decreased (2.00 vs 1.86; $P < 0.001$), while A260/A280 remained unchanged ($P = 0.711$). DNA concentration slightly increased (40.31 vs 41.46 ng/μL; $P < 0.001$), indicating spectrophotometric concentration did not reflect DNA integrity decline. PCR genotyping showed no significant deterioration, with preserved amplification success (100.0% vs 99.6%; $P = 1.000$), no significant increase in repeat testing (0.0% vs 2.24%; $P = 0.063$), and genotype concordance of 99.6%.

Conclusion: Long-term refrigerated storage was associated with reduced DNA integrity and changes in purity parameters, but no meaningful performance loss was observed in this PCR-based genotyping assay. These findings support robustness of the short-amplicon assay to prolonged refrigerated storage, but should not be generalized to other molecular applications without further validation.

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J-08

Impact of age on presentation and molecular response to therapy in chronic myeloid leukemiaToni Božac¹, Monika Kolundžić¹, Ivana Lapić^{1,2}, Jadranka Jerković¹, Marijana Jurak¹, Antonio Vrselja¹, Željka Vujin¹, Margareta Radić Antolić¹¹Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia²Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm defined by presence of Philadelphia chromosome and BCR::ABL1 fusion gene. Younger patients account for a minority of cases and data on disease biology and therapy response remain conflicting. This study was aimed to evaluate disease characteristics and molecular response between younger and older populations.

Materials and methods: Data from 48 CML patients were retrospectively analyzed. Patients were divided by age at diagnosis into younger (≤ 30 years) and older (≥ 31 years) groups. Data included complete blood count with differentials, chromosomal aberrations at diagnosis and quantitative BCR::ABL1 transcript level at diagnosis and in follow-up (3, 6 and 12 months). Molecular response was defined according to 2025 European LeukemiaNet CML recommendations. Continuous variables were compared using Mann-Whitney U test while categorical variables were compared using Fisher's exact test.

Results: Among 48 patients, 10 patients (21%) were in the younger group and 38 patients (79%) were in the older group. Younger patients had significantly higher leukocyte counts $119.4 \times 10^9/L$ (IQR: 67.6-183.7) compared to $62.0 \times 10^9/L$ (IQR: 30.2-122.6) in older group ($P = 0.043$). No significant changes were observed in erythrocytes ($P = 0.151$), thrombocytes ($P = 0.960$), basophils ($P = 0.585$) or presence of additional cytogenetic changes at time of diagnosis ($P = 1.000$). There was no significant difference in molecular response at 3-month ($P = 0.425$) or 6-month ($P = 1.000$) follow-up. At 12-month follow-up, there was significant difference: 2/10 (20%) younger patients achieved favourable responses while 8/10 (80%) remained in warning category, on the contrary 23/38 (61%) older patients reached a favourable response, 13/38 (34%) were in warning category and 2/38 (5%) in unfavorable category ($P = 0.014$).

Conclusion: Younger patients presented with higher leukocyte counts and lower rates of molecular response at the 12-month follow-up, suggesting higher disease burden and delayed achievement of molecular response. Additional genomic analysis may clarify age-related differences of CML and optimize treatment strategies for younger patients.

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J-09

Comprehensive molecular profiling of FLT3 mutations: prevalence of atypical mutationsToni Božac¹, Monika Kolundžić¹, Ivana Lapić^{1,2}, Jadranka Jerković¹, Marijana Jurak¹, Antonio Vrselja¹, Željka Vujin¹, Margareta Radić Antolčić¹¹Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia²Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Introduction: FMS-like tyrosine kinase 3 (FLT3) mutations represent a common molecular subtype of acute myeloid leukemia (AML) and are associated with poor prognosis. Majority of FLT3 mutations include: internal tandem duplications (ITD) in the juxtamembrane domain (JMD) and point mutations in tyrosine kinase domain (TKD). Next generation sequencing (NGS) enabled detection of novel FLT3 mutations. This study was aimed to analyze the spectrum of FLT3 mutations, focusing on atypical FLT3 mutations.

Materials and methods: We retrospectively analyzed NGS data from patients with AML. Only patients with detected FLT3 mutations were included. FLT3 variants were classified into 3 categories: FLT3-ITDs, D835 mutations in TKD2 and atypical FLT3 mutations. Atypical mutations were further subclassified according to the functional domain they occur: JMD, TKD1, TKD2 and extracellular domain. Descriptive statistics were used to analyze data.

Results: A total of 50 mutations were detected across 44 patients. Among detected FLT3 mutations, 20 (40.0%) mutations were ITD, 11 (22.0%) mutations were D835 mutations in TKD2, while 19 (38.0%) mutations were atypical FLT3 mutations. FLT3-ITD mutations were exclusively located in the JMD. Among atypical FLT3 mutations, 3/19 were present in the TKD1, 7/19 were present in TKD2, 5/19 were present in the JMD and 4/19 were located in the extracellular domain. Most common TKD1 mutations were: N676K and A680V, while TKD2 mutations included: I836del, D839G, Y842C, I867S and N841K. Point mutations located in the JMD included: Y572C, Q577E, V579G and V592A. Mutations detected in extracellular regions were: V194M, D342N and S451F.

Conclusion: In our patient cohort we detected a significant number of atypical FLT3 mutations present outside of known hotspot regions. Since emerging data suggest clinical significance and therapeutic effectiveness for detected atypical mutations, comprehensive NGS analysis is essential for precise and accurate diagnosis of patients with AML.

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J-10

Optimisation of plasma and RNA input volumes for circulating microRNA expression analysis in acute myeloid leukaemia

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Introduction: MicroRNAs (miRNAs) in plasma are promising biomarkers for acute myeloid leukaemia (AML). This study aimed to evaluate the influence of plasma and RNA input volumes on the quality and reliability of plasma miRNA expression analysis.

Materials and methods: The study included 10 plasma samples enrolled in the HRZZ-IP-2025-02-5566 project: four paired AML patient samples collected at two time points and two healthy control samples. RNA was isolated from 200 and 400 µL plasma using the MiRNeasy Serum/Plasma Advanced Kit (Qiagen). Expression of target (miR-223, miR-181a-3p, miR-181b-5p, miR-217, miR-22, miR-133, miR-222), reference (miR-16-5p, miR-103a-3p, miR-191-5p), and control miRNAs (UniSp-100, UniSp-101, UniSp6, miR-451a, miR-23a-3p) was analysed by RT-qPCR using the miRCURY RT LNA kit (Qiagen) with 2 and 4 µL of RNA input and the miRCURY LNA SYBR GREEN PCR Kit (Qiagen) on the QuantStudio 7 Flex system (Applied Biosystems). The Wilcoxon signed-rank test was used for statistical analysis (MedCalc Software Ltd, v23.4.8) with $P < 0.05$ considered statistically significant.

Results: No statistically significant differences were observed between 200 and 400 µL plasma input volumes. A statistically significant difference between the 2 and 4 µL RNA input volumes for RT was observed in samples isolated from 200 µL of plasma for miR-133 ($P = 0.002$), miR-181b-5p ($P = 0.010$), miR-103a-3p ($P = 0.002$), miR-191-5p ($P = 0.002$), miR-16-5p ($P = 0.002$), miR-22 ($P = 0.002$), miR-222 ($P = 0.002$), miR-223 ($P = 0.002$), miR-23a-3p ($P = 0.002$), miR-451a ($P = 0.002$), and UniSp101 ($P = 0.004$), and similarly in samples isolated from 400 µL of plasma for miR-133 ($P = 0.008$), miR-103a-3p ($P = 0.002$), miR-23a-3p ($P = 0.002$), miR-451a ($P = 0.002$), miR-16-5p ($P = 0.002$), miR-22 ($P = 0.002$), miR-223 ($P = 0.002$), miR-191-5p ($P = 0.006$), and miR-222 ($P = 0.004$). In both cases, Ct values were lower when 4 µL input volume was applied.

Conclusion: These results suggest that plasma input volume has minimal impact on miRNA detection, whereas increasing RNA input volume for RT improves the sensitivity and reliability of plasma miRNA analysis in AML samples.

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J-11

Comparison of two commercial microRNA extraction kits for plasma samples

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Introduction: This study aimed to compare the performance of two commercially available miRNA extraction assays for plasma samples using reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Materials and methods: Blood samples from 10 AML patients enrolled in the HRZZ-IP-2025-02-5566 project were collected in EDTA tubes. Plasma was isolated by sequential centrifugation at 1900xg and 16,000xg for 10 min each at + 4 °C. miRNA was extracted from 200 µL plasma using the miRNeasy Serum/Plasma Kit (Qiagen, Germany) and miRNeasy Serum/Plasma Advanced Kit (Qiagen, Germany). RT was performed with the miRCURY RT LNA Kit (Qiagen, Germany) using 2 µL of RNA. Expressions of UniSp-100, UniSp-101, UniSp6, miR-451a, miR-23a-3p, miR-103a-3p, and miR-191-5p were determined on the QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, USA) with the miRCURY LNA SYBR GREEN PCR Kit (Qiagen, Germany). Statistical analysis was performed in MedCalc (version 23.24.8) using the Wilcoxon signed-rank test, with $P < 0.05$ considered statistically significant.

Results: Statistically significant differences were observed for the endogenous miRNAs miR-23a-3p, miR-451a, and miR-103a-3p. Median Ct values for the miRNeasy Serum/Plasma Kit *versus* the miRNeasy Serum/Plasma Advanced Kit were 28.24 (27.73–29.34) vs 26.38 (25.32–27.70) for miR-23a-3p ($P = 0.037$), 20.28 (19.04–21.91) vs 18.54 (17.42–19.41) for miR-451a ($P = 0.049$), and 28.98 (27.73–30.26) vs 27.45 (26.09–28.30) for miR-103a-3p ($P = 0.049$). A statistically significant difference was also observed for UniSp6, with median Ct values of 19.68 (19.58–19.76) for the miRNeasy Serum/Plasma Kit and 19.20 (18.85–19.27) for the miRNeasy Serum/Plasma Advanced Kit ($P = 0.010$). No statistically significant differences were observed for UniSp-100, UniSp-101, or miR-191-5p ($P = 0.889$, $P = 0.160$, $P = 0.106$, respectively).

Conclusion: Although the results show comparable performance for UniSp-100, UniSp-101, and miR-191-5p, lower Ct values for the endogenous miRNAs miR-23a-3p, miR-451a, and miR-103a-3p indicate slightly higher sensitivity of the miRNeasy Serum/Plasma Advanced Kit.

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J-12

Comparison of digital multiplex ligation-dependent probe amplification (digital MLPA) and oligonucleotide array comparative genomic hybridization with SNP analysis (aCGH+SNP) in patients with acute lymphoblastic leukemia

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Introduction: Acute lymphoblastic leukemia (ALL) is the most common hematologic malignancy in childhood and also occurs in adults. Detection of chromosomal abnormalities is essential for prognosis assessment and therapy selection. Digital multiplex ligation-dependent probe amplification (digital MLPA) and oligonucleotide array comparative genomic hybridization with single-nucleotide polymorphism (SNP) analysis (aCGH+SNP) are newer methods for detecting chromosomal abnormalities. This study aimed to compare digital MLPA and aCGH+SNP in DNA samples from patients with ALL.

Materials and methods: The study included 22 DNA samples isolated from bone marrow of patients diagnosed with ALL between January 2022 and December 2025. DNA extraction was performed using the QIAcube automated system. Digital MLPA and aCGH analysis were carried out according to manufacturers' protocols (MRC Holland dMLPA version 009, 2025; Agilent Protocol version 8.0, 2019). Copy number alterations of ALL-associated genes were analyzed using the corresponding software platforms, and results obtained by the two methods were compared.

Results: Both methods successfully detected chromosomal abnormalities associated with ALL and classified patients into appropriate risk stratification groups. The aCGH+SNP method demonstrated higher resolution and identified additional genomic alterations and regions of loss of heterozygosity, while digital MLPA enabled faster targeted analysis of specific genes. Agreement between the methods in patient stratification showed perfect concordance (Cohen's $\kappa = 1.00$), with 100% agreement across all 22 patients.

Conclusion: Both digital MLPA and aCGH+SNP demonstrated high diagnostic value in the genetic analysis of ALL. Digital MLPA represents a rapid and cost-effective method for targeted detection of known abnormalities, whereas aCGH+SNP enables more comprehensive genome analysis and identification of additional chromosomal alterations. Combined use of both methods may improve genetic characterization and optimize the diagnostic approach in ALL.

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J-13

Effect of second elution and plasma input volume on the extraction efficiency of circulating cell-free DNA in patients with acute myeloid leukaemia

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Introduction: The quantity of circulating cell-free DNA (ccfDNA) is essential for downstream analyses and the assessment of its diagnostic potential in malignant diseases. This study evaluated the effect of an additional second column elution step and plasma input volume on the extracted ccfDNA quantity normalised *per* mL of plasma in patients with acute myeloid leukaemia (AML).

Materials and methods: ccfDNA was extracted from 1 and 4 mL plasma samples of 10 AML patients enrolled in the HRZZ-IP-2025-02-5566 project using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany) with an additional second column elution step. ccfDNA concentrations were determined using the Qubit dsDNA HS Assay Kit (Invitrogen, USA) on the fluorometer DS-11 FX (DeNovix, USA). ccfDNA quantities *per* mL of plasma were calculated separately for the first eluate and for the combined first and second eluates (total ccfDNA quantity). Results are presented as median and interquartile range. Statistical analysis of paired results was performed using the Wilcoxon signed-rank test (MedCalc Software Ltd, v20.305, Ostend, Belgium), with $P < 0.050$ considered statistically significant.

Results: Statistically significant differences in ccfDNA quantities *per* mL of plasma were observed between the first eluate and combined eluates at both examined plasma input volumes (1 mL: 26.05 (8.50-31.20) vs 26.94 (8.86-32.74) ng/mL, respectively, $P = 0.018$; 4 mL: 16.50 (5.38-23.75) vs 18.72 (5.48-24.78) ng/mL, respectively, $P = 0.002$). No statistically significant difference in total ccfDNA quantity *per* mL of plasma was observed between extractions performed from 1 and 4 mL plasma samples ($P = 0.106$).

Conclusion: Residual ccfDNA remained on the column after the first elution regardless of plasma input volume, suggesting that the inclusion of an additional second elution step may be beneficial when ccfDNA quantity *per* mL of plasma is evaluated as a potential biomarker for AML. Plasma input volume did not significantly affect total extracted ccfDNA quantity *per* mL of plasma.

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J-14 (Oral presentation)

Integrated genomic profiling of ovarian cancer reveals low tumor mutational burden, microsatellite stability, and actionable alterationsTadej Pajic^{1,2}, Aleš Maver¹, Urška Kotnik¹, Borut Kobal³, Borut Peterlin¹¹Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia²Clinical Biochemistry, Faculty of Medicine, University of Maribor, Maribor, Slovenia³Department of Gynecology, Division of Gynecology and Obstetrics, University Medical Centre Ljubljana, Ljubljana, Slovenia

Introduction: Ovarian cancer remains a leading cause of gynecologic cancer mortality and is characterized by marked molecular heterogeneity. Comprehensive genomic profiling enables simultaneous assessment of clinically relevant genomic alterations and biomarkers associated with therapeutic stratification. We hypothesized that integrated genomic profiling could identify recurrent molecular alterations and relevant biomarkers in ovarian cancer. The aim of this study was to characterize genomic alterations, tumor mutational burden (TMB), and microsatellite instability (MSI) status using targeted next-generation sequencing.

Materials and methods: Twenty-five fresh frozen ovarian cancer samples were analyzed using the TruSight Oncology 500 assay. DNA-based profiling included detection of single nucleotide variants, insertions/deletions, and copy number alterations. TMB and MSI status were determined using the standard bioinformatics pipeline. Descriptive statistical analysis was performed using median values, ranges, and frequency distributions of genomic alterations and biomarker status.

Results: Comprehensive genomic profiling was successfully performed in all samples (25/25; 100%). The median TMB was 3.1 mut/Mb (range 0.0–11.8). Most tumors were classified as TMB-low (23/25; 92%), while 2/25 (8%) showed intermediate TMB levels. All tumors were microsatellite stable (25/25; 100%). TP53 was the most frequently altered gene (20/25; 80%), followed by PIK3CA and KRAS (4/25; 16% each). Recurrent alterations in homologous recombination-related genes included BRCA1 (3/25; 12%), BRCA2 (2/25; 8%), PALB2 (2/25; 8%), and RAD51C (1/25; 4%). Recurrent copy number amplifications most frequently involved AKT2, CCNE1, and KRAS (3/25; 12% each).

Conclusion: This cohort demonstrated a consistent molecular profile characterized by low TMB, absence of MSI-high tumors, and frequent TP53 alterations. Concurrent alterations in homologous recombination-related genes and PI3K pathway components highlighted potential opportunities for targeted therapies, including PARP inhibitors, emphasizing the clinical utility of comprehensive genomic profiling. Further integration of homologous recombination deficiency and genomic scar analyses may improve molecular stratification and support more personalized therapeutic decision-making in ovarian cancer.

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J-15

Impact of ABCB1 and CES1 variants on clinical outcomes of dabigatran therapyIvana Prpic¹, Jozefina Palic^{2,3}, Tamara Božina², Livija Šimičević^{2,3}, Lana Ganoci^{3,4}¹Polyclinic Breyer, Zagreb, Croatia²Department for Chemistry and Biochemistry, School of Medicine, University of Zagreb, Zagreb, Croatia³Division for Pharmacogenomics and Therapy Individualization, Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia⁴Department of Pharmacology, University of Zagreb School of Medicine, Zagreb, Croatia

Introduction: Dabigatran etexilate is activated by CES1, while ABCB1 affects its absorption. Previous studies have demonstrated interindividual variability in dabigatran plasma concentrations associated with certain ABCB1 and CES1 variants; however, their clinical relevance remains unclear. This study investigated the impact of those variants and haplotypes, together with non-genetic factors, on dabigatran clinical outcomes.

Materials and methods: Presented results are preliminary data of a larger PGx-CardioDrug cohort study. Adult participants receiving dabigatran etexilate (150/110 mg bid) for ≥ 3 months were included. Bleeding-associated and thromboembolic events were evaluated as clinical outcomes. Subjects were genotyped for four ABCB1 (rs1128503, rs2032582, rs1045642, rs4148738) and two CES1 variants (rs2244613, rs8192935) using real-time PCR (ABI-7500; LightCycler). Associations between variants and outcomes were analysed using standard genetic models. Clinically relevant data were collected from medical records. Drug-drug interactions were analysed using UpToDate Lexidrug. Statistical analysis was performed using Fisher's exact test.

Results: Of 85 included participants (median age 68; 35 females/50 males), 22 reported bleeding associated and 9 thromboembolic outcomes. CES1 rs2244613 deviated from HWE and was excluded from analysis. No significant associations were observed between variants or haplotypes and clinical outcomes in any genetic model. The recessive model for ABCB1 rs1128503 showed a non-significant trend toward increased bleeding risk ($P = 0.062$). Significant associations with bleeding risk were observed for female sex ($OR = 6.2$; $P = 0.008$), reduced dabigatran dose ($OR = 6.1$; $P = 0.001$), impaired renal function ($OR = 5.3$; $P = 0.003$), older age ($OR = 6.1$; $P = 0.001$), and drug-drug interactions affecting dabigatran exposure ($OR = 0.3$; $P = 0.043$). Prior thromboembolic events were significantly associated with thromboembolic outcomes ($OR = 8.7$; $P = 0.031$).

Conclusion: No significant associations were observed between ABCB1 and CES1 variants and dabigatran-related clinical outcomes, although ABCB1 rs1128503 showed a borderline trend toward increased bleeding risk. Several non-genetic factors showed significant association with adverse outcomes. Larger cohort analysis is needed to clarify the clinical role of pharmacogenetics in dabigatran-treated patients.

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J-16 (Oral presentation)

Impact of CYP2C19, CYP2B6, ABCB1 and 5-HTTLPR pharmacogenetic variants on sertraline pharmacokinetics, therapeutic response and adverse drug reactions in the treatment of depression

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Introduction: Sertraline is a widely prescribed selective serotonin reuptake inhibitor metabolised by cytochrome P450 (CYP) enzymes (CYP2C19, CYP2B6, CYP2D6, CYP3A4, CYP2C9), primarily into N-desmethylsertraline. Also, it is a substrate of ABCB1 and ABCG2 drug transporters. This pilot study evaluated the influence of pharmacogenetic variants on sertraline pharmacokinetics, therapeutic response and adverse drug reactions (ADRs) in patients with depression.

Materials and methods: This study included psychiatric patients with depression treated with sertraline and concomitant benzodiazepines. Participants were genotyped for pharmacogenetic panel including ABCB1, ABCG2, COMT, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5 and 5-HTTLPR. Sertraline and N-desmethylsertraline serum concentrations were quantified by LC–MS/MS to calculate area under the drug concentration-time curve (AUC) values, while therapeutic response was evaluated using patient outcome reports (POR).

Results: Forty-five patients (29 women, 16 men), median age 41 years (18–68), receiving sertraline (20–100 mg/day) and concomitant benzodiazepines were included. Sertraline AUC_{0–6} (ng × h/mL) were lower in CYP2B6 intermediate metabolizers (IM) than normal metabolizers (NM) (7.3 vs. 11.1), while CYP2C19 intermediate/poor metabolizers (IM+PM) showed higher exposure than rapid/ultrarapid metabolizers (RM+UM) (11.6 vs. 8.3). ABCB1 c.3435T allele carriers had higher sertraline concentrations than C allele carriers (11.2 vs 7.2). For N-desmethylsertraline, CYP2B6 IM patients had lower AUC_{0–6} values than NM (12.2 vs. 19.5), whereas CYP2C19 IM/PM patients showed higher exposure than RM/EM (21.1 vs. 14.9). In multivariate analysis, the ABCB1 low-function phenotype was associated with increased sertraline exposure ($\beta = 3.13$, $P = 0.036$). Patients with normal SERT function demonstrated greater motivation improvement. Common ADRs included dry mouth, dizziness, increased appetite and sleep disturbances.

Conclusion: Preliminary findings indicate that CYP2C19, CYP2B6 and ABCB1 variants modulate sertraline and N-desmethylsertraline exposure, while SERT variability may influence therapeutic response. These results support the potential role of pharmacogenetic-guided sertraline therapy in depression.

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K Point-of-care testing

K-01

Verification of point-of-care Radiometer ABL90 Flex analyzer in Karlovac General Hospital

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Introduction: The availability of rapid, on-the-spot biochemical evaluations for monitoring vital parameters enables faster identification of severe illness and poor outcomes, allowing prompt interventions in the Intensive Care Unit (ICU). This study aimed to evaluate the analytical specifications of point-of-care ABL90 Flex analyzer in the ICU for acid-base status parameters, sodium, potassium, chloride, ionized calcium, glucose, lactate and hemoglobin measurements and its comparison with the core laboratory.

Materials and methods: Repeatability, day-to-day and total precision were determined using two levels of quality control following CLSI guidelines EP15-A3 on the ABL90 Flex analyzer (Radiometer, Bronshoj, Denmark). Results from the arterial blood samples collected in lithium heparinized syringes (N = 40) evaluated on ABL90 Flex analyzer were compared with the results from the core laboratory blood-gas analyzer Rapidlab 1265 (Siemens, Erlangen, Germany), EDTA-whole blood samples on the hematology analyzer XN550 (Sysmex, Kobe, Japan), serum and sodium fluoride plasma samples on the chemistry analyzer Alinity ci (Abbott, Abbott Park, USA). Method comparison was performed using the Bland-Altman and Passing-Bablok regression analysis in the MedCalc program v10.0.2.0 (MedCalc Software Ltd, Ostend, Belgium).

Results: All analytes met the manufacturer's acceptance criteria for precision testing except pO₂ in low concentration range (repeatability, day-to-day and total precision coefficients of variations were 6.4, 3.8 and 6.9% respectively). Passing-Bablok regression indicated proportional and constant difference for pCO₂ and pO₂, constant difference for pH, sodium, potassium, ionized calcium and no proportional nor constant difference for lactate, glucose, hemoglobin and chloride. Bland-Altman analysis yielded clinically significant relative bias for potassium (6.9%, 95% CI: 5.4 to 8.5) due to sample type differences, while relative biases for other analytes were not clinically significant.

Conclusion: The ABL90 Flex analyzer met most of the manufacturer's analytical specifications. Method comparison verified that concentrations for all examined analytes except potassium can be measured interchangeably in ICU and in core laboratory.

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K-02

The role of laboratory supervision in POCT quality control: a case report of discrepant glucose values

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Introduction: Glucose is among the most frequently measured laboratory parameters and is commonly measured using point of care testing (POCT) devices such as blood gas analyzers and glucometers. Since POCT enables rapid clinical decision-making, maintaining measurement accuracy requires continuous laboratory oversight and quality control (QC) monitoring. Systematic evaluation of QC trends can reveal early signs of analytical deterioration before patient safety is compromised.

Materials and methods: A clinically significant discrepancy in glucose measurements between the ABL90 Flex Plus blood gas analyzer (Radiometer, Denmark) and the Contour XS glucometer (Bayer, Germany) in a neonatal intensive care unit was investigated. Patient results, internal QC data at three concentration levels and Levey–Jennings charts were retrospectively analyzed.

Results: A discrepancy between glucose values obtained by ABL90 Flex Plus (11 mmol/L) and Contour XS (17 mmol/L) was promptly reported by the clinician. Although all QC results remained within acceptable limits, retrospective review of Levey–Jennings charts revealed a gradual downward trend in level 3 QC values over four days on the ABL90 Flex Plus. This was indication of reduced sensor sensitivity. Following replacement of the sensor cassette, comparability between devices was restored within predefined acceptance criteria.

Conclusion: This case demonstrates that QC results may remain within acceptable limits despite the presence of systematic bias. In POCT settings, clinicians typically rely on sensor status indicators rather than QC trend analysis, which may delay recognition of analytical issues. Early detection of performance changes is essential to maintain confidence in POCT systems and ensure reliable results. Continuous QC trend monitoring, supported by laboratory expertise and effective communication with clinical staff, enables timely intervention and patient safety. This case underscores the critical importance of close collaboration and continuous laboratory oversight in POCT quality management.

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K-03

Comparison of total hemoglobin measurements between the Radiometer ABL90 FLEX blood gas analyzer and the Sysmex hematology analyzer

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Introduction: Point-of-care blood gas analyzers are increasingly used for rapid hemoglobin (Hb) assessment in emergency and critical care settings. However, analytical agreement with standard hematology analyzers must be verified before interchangeable clinical use. This study aimed to compare Hb measurements obtained using the Radiometer ABL90 FLEX blood gas analyzer (ABL) and the Sysmex hematology analyzer.

Materials and methods: Patient samples were analyzed in parallel using EDTA whole blood on the Sysmex hematology analyzer and blood gas whole blood samples on the ABL analyzer, reflecting routine use of both analytical systems. Method comparison was performed using Passing-Bablok regression and Bland–Altman analysis, while differences between paired measurements were assessed using the Wilcoxon signed-rank test. Statistical analyses were performed using MedCalc software. A P value < 0.05 was considered statistically significant. The desirable specification for bias was set at 1.7% according to the EFLM biological variation database.

Results: A total of 80 paired Hb measurements were included. Median (IQR) Hb concentration was 100 g/L (93–134) for Sysmex and 116 g/L (88–123) for ABL. Passing-Bablok regression revealed no statistically significant systematic or proportional difference between methods ($ABL = 0.783$ (95% CI: - 3.125 to 5.500) + 1.043 (95% CI: 1.000 to 1.083) $\times$ Sysmex). Bland–Altman analysis demonstrated a mean bias of - 7% (95% CI: - 9% to - 5%), exceeding the desirable EFLM criteria. Wilcoxon signed-rank testing showed a statistically significant difference between paired measurements ($P < 0.001$).

Conclusions: Although Passing-Bablok regression demonstrated no significant systematic or proportional differences between the analyzers, Bland–Altman and Wilcoxon analyses revealed clinically and statistically significantly higher Hb measurements on the ABL analyzer compared with Sysmex. Therefore, Hb results obtained using the ABL90 FLEX and Sysmex analyzers should not be considered interchangeable without prior verification and careful clinical interpretation.

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K-04

Comparison of urea and creatinine measurements between the Stat Profile Prime Plus and Roche Cobas c503 analyzers

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Introduction: The Stat Profile Prime Plus is a point-of-care analyzer that provides rapid measurement of blood gases, electrolytes, metabolites, CO-oximetry, and calculated parameters. This study aimed to compare whole blood urea and creatinine results obtained on the Prime Plus analyzer with routinely measured serum urea and creatinine results obtained on the Roche Cobas c503 analyzer.

Materials and methods: Urea and creatinine were determined in heparinized whole blood samples on the Prime Plus analyzer using methods based on enzymatic reactions and potentiometry. Results were compared with parallel serum samples analyzed on the Cobas c503 analyzer using an enzymatic method for urea and the Jaffe method for creatinine. Method comparison was performed using Passing-Bablok regression and Bland-Altman analysis. Statistical analyses were performed using MedCalc software.

Results: The study included 68 samples. Median (IQR) urea concentrations were 12.3 mmol/L (6.1-19.0) and 11.9 mmol/L (6.0-17.9) for Cobas and Prime Plus, respectively, while creatinine concentrations were 107 μ mol/L (66-203) and 77 μ mol/L (57-162), respectively. Passing-Bablok regression revealed no significant systematic or proportional difference for urea (Prime Plus = - 0.125 (95% CI: - 0.559 to 0.334) + 1.042 (95% CI: 0.979 to 1.107) $\times$ Cobas). In contrast, creatinine comparison demonstrated a significant proportional difference between methods (Prime Plus = - 1.210 (95% CI: - 9.208 to 7.641) + 0.849 (95% CI: 0.785 to 0.899) $\times$ Cobas). Bland-Altman analysis showed a mean bias of 1.0% for urea and 20.0% for creatinine.

Conclusions: Urea measurements obtained on the Prime Plus analyzer showed good agreement with Cobas c503. In contrast, creatinine measurements demonstrated substantial proportional bias, with lower creatinine values observed on the Prime Plus analyzer. Therefore, creatinine results obtained using these analytical systems should not be considered interchangeable.

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L Sports medicine

L-01

Effects of regular summer recreational diving on erythropoietin and red blood cell parameters

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Introduction: Hyperoxia during recreational scuba diving may induce oxidative erythrocyte damage and may also affect erythropoietin (EPO) production, a response often attributed to the hyperoxic-hypoxic paradox. This study investigated the effects of regular summer recreational diving on EPO concentrations and red blood cell (RBC) parameters.

Materials and methods: The study included 12 recreational divers (10 males, 2 females), median age 46 years (range 22-56 years), with more than 20 dives during the 21-week summer diving season. Divers participated in organized club dives once or twice weekly under habitual recreational open-water conditions. Blood samples were collected in early May (pre-season) and late September (post-season) in the morning under standardized conditions. Serum EPO concentrations were measured using an Immulite 2000 Xpi analyzer (Siemens Healthcare Diagnostics, Flanders, USA), while RBC parameters were determined using a DxH 900 hematology analyzer (Beckman Coulter, Brea, USA). Differences between pre- and post-season measurements were evaluated using the Wilcoxon signed-rank test. Associations between the number of dives and percentage changes in the examined parameters were assessed using Spearman's rank-order correlation (ρ). Statistical analysis was performed using IBM SPSS Statistics. $P < 0.05$ was considered statistically significant.

Results: The median (range) number of dives was 26 (20-41). After the summer diving season, no significant changes were observed in the examined parameters except for increases in MCH and MCHC ($P = 0.007$ and $P = 0.009$, respectively). A high positive association was found between the number of dives and percentage changes in EPO concentrations ($P = 0.002$, $\rho = 0.80$), while no significant associations were observed for the other examined parameters.

Conclusion: Regular recreational scuba diving once or twice weekly for 21 weeks did not affect EPO concentrations or most RBC parameters. The positive association between the number of dives and percentage changes in EPO suggests that more frequent diving may influence EPO concentrations.

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L-02

Insulin sensitivity across the menstrual cycle: a comparison between athletes and recreationally active women

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Introduction: Insulin sensitivity fluctuates across the menstrual cycle and may be further influenced by training status, yet differences between athletes and recreationally active women remain insufficiently understood.

Materials and methods: A total of 33 healthy, eumenorrheic women aged 18-30 years were recruited and categorized as athletes (N = 18) or non-athletic controls (N = 15). Menstrual cycle phase was determined based on the first day of menstrual bleeding, documented cycle length from preceding months, and endocrinological evaluation of luteinizing hormone, estradiol, and progesterone concentrations. Serum insulin concentrations were measured using an electrochemiluminescence immunoassay on the Roche Cobas e402 analyzer. Glucose concentrations were assessed spectrophotometrically using the Abbott Alinity c analyzer. Insulin resistance and β -cell function were estimated using HOMA-IR and HOMA-B indices, calculated with standard formulas via the HOMA2 Calculator (Version 2.2.3). Differences across menstrual cycle phases were analyzed using the Friedman test for paired samples (MedCalc® Statistical Software, version 23.4.9), with statistical significance at $P < 0.05$.

Results: In athletes, significant variations across the menstrual cycle were observed for insulin ($P = 0.006$), glucose ($P = 0.007$), insulin sensitivity ($P = 0.004$), and insulin resistance ($P = 0.004$), with values indicating reduced insulin sensitivity and increased insulin resistance in the luteal phase; no significant changes were found in β -cell activity ($P = 0.149$). No significant phase-dependent differences were observed in the control group for insulin ($P = 0.693$), insulin sensitivity ($P = 0.428$), insulin resistance ($P = 0.428$), or β -cell activity ($P = 1.000$). Glucose showed a non-significant trend toward variation ($P = 0.083$).

Conclusions: Training status significantly influences metabolic responses across the menstrual cycle, with athletes exhibiting reduced insulin sensitivity and increased insulin resistance during the luteal phase, whereas controls show no meaningful phase-dependent changes. These findings suggest that regular training may amplify cyclical metabolic fluctuations without affecting β -cell function.

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L-03

Menstrual cycle phase and its impact on strength and power performance

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Introduction: We aimed to assess the strength-related sports performance of female athletes across menstrual cycle phases and compare it with that of recreationally active women.

Materials and methods: A total of 33 healthy, eumenorrheic women aged 18–30 years were recruited and categorized as athletes (N = 18) or non-athletic controls (N = 15). Menstrual cycle phase was determined based on the first day of menstrual bleeding and endocrinological evaluation of luteinizing hormone, estradiol, and progesterone concentrations. Motor skill testing included: isokinetic measurements of peak torque of the extensors (PTE) and flexors of the upper leg and the strength ratios of these muscle groups; hand grip strength measured with the Takei and Jamar dynamometers; explosive power measured by sprint and jump; and strength endurance measured by hold-on in the high pull-up. Differences across menstrual cycle phases were analyzed using the Friedman test for paired samples (MedCalc Statistical Software, version 23.4.9), with statistical significance at $P < 0.05$.

Results: During ovulation, athletes showed significantly higher values in PTE at 60° ($P = 0.038$), average power strength of the extensors at 60° ($P = 0.044$), and at 180° ($P = 0.017$). In the luteal phase, significant differences were also observed for PTE at 60° ($P = 0.027$) and average strength of the extensors at 60° ($P = 0.022$), with athletes again demonstrating higher values. Hand grip strength and sprint performance during ovulation approached statistical significance, indicating a consistent trend toward better neuromuscular performance in athletes.

Conclusions: Athletes demonstrated superior strength-related performance compared to controls, with the most pronounced differences occurring during ovulation and, to a lesser extent, in the luteal phase. These findings suggest that menstrual cycle phase, particularly ovulation, may influence neuromuscular performance to a greater extent in athletes than in non-athletes and should be considered in training and performance, possibly allowing timing manipulation for competition days.

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L-04

Complete blood count stability differs between female athletes and the general population during delayed analysisAna Nikler^{1,2}, Vanja Radišić Biljak^{1,2}¹Department of Medical Laboratory Diagnostics, University Hospital Sveti Duh, Zagreb, Croatia²Department of Sport and Exercise Medicine, Faculty of Kinesiology, University of Zagreb, Zagreb, Croatia

Introduction: Delayed complete blood count (CBC) analysis is common in transport-dependent workflows, including sports medicine. This study aimed to evaluate the time- and temperature-dependent stability of CBC parameters, with a specific focus on comparing stability patterns between female athletes and the general population.

Materials and methods: Venous blood was collected from 40 adult women from the general population and 75 female athletes into two K2EDTA tubes. One tube was stored at 20 °C and the other one at 4 °C. CBC parameters (including reticulocytes) were measured on a Siemens ADVIA 2120i (Erlangen, Germany) after 0, 8, 48, 72 hours. The OFF-hr score was calculated as $\text{hemoglobin (g/L)} - 60 \times \sqrt{\text{reticulocyte percentage}}$. Stability was expressed as the mean percent bias relative to baseline (0 h), with 95% confidence intervals, and assessed against the maximum permissible difference (MPD) derived from biological variation.

Results: At 20 °C, both groups showed similar stability. The earliest between-group difference was mean corpuscular volume (MCV) after 8 hours (athletes 0.9% vs general population 2.1%; MPD 1.5%). At 4 °C, female athletes remained stable for up to 72 hours across all assessed parameters, except mean platelet volume, which increased early (5.4% bias at 8 hours) and continued to drift. In the general population at 4 °C, stability was shorter for selected indices (MCV stable up to 8 hours; leukocytes, hematocrit, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were stable up to 48 hours). For OFF-hr score, deviations were similar across time points at 4 °C, whereas at 20 °C the smallest deviation occurred at 8 hours (5.2% general population; 4.3% athletes) and increased to 72 hours (42.1% and 52.0%, respectively), consistent with declining reticulocytes during room-temperature storage.

Conclusion: CBC stability is population- and temperature-dependent. Under cold-chain conditions, most CBC parameters in female athletes remain stable up to 72 hours, supporting acceptance of delayed CBC testing in sports medicine.

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L-05

Circulating calprotectin response to acute scuba diving in recreational divers

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Introduction: Circulating calprotectin (circCalp) is a protein secreted by activated neutrophils and monocytes during inflammatory reactions. It has also been increasingly recognized as a biomarker of exercise-induced inflammation and physiological stress in sports. This study aimed to investigate the effect of a single scuba dive on circCalp levels in a group of recreational scuba divers.

Materials and methods: The study included 23 recreational divers (21 males and 2 females), median aged 48 years (range 18-58). The experimental dive took place in early May, in the morning hours, with a maximum depth of 30 meters and a total dive duration of 30 minutes. Blood samples were collected immediately before the dive (pre-dive), immediately after the dive (post-dive 1), and three hours after the dive (post-dive 2), and were centrifuged within 30 minutes of collection. CircCalp concentrations were determined using the Bio-Flash analyzer (Biokit S.A., Spain). Results were analyzed using the Friedman test with Dunn's *post-hoc* pairwise comparisons. Statistical analysis was performed using IBM SPSS software. P-value < 0.05 was considered statistically significant.

Results: Median (IQR) circCalp values (ng/mL) for pre-dive, post-dive 1, and post-dive 2 were 96 (62-158), 160 (84-219), and 166 (95-231), respectively. The Friedman test revealed a statistically significant difference between the measurements ($P < 0.001$). A significant increase in circCalp concentrations was observed in post-dive 1 compared to pre-dive ($P < 0.001$), while no significant difference was found between post-dive 1 and post-dive 2 ($P = 1.000$).

Conclusion: A single scuba dive induces a significant increase in circulating calprotectin levels, suggesting acute activation of the innate immune system. The absence of further increase three hours post-dive indicates that this response occurs rapidly after exposure and then stabilizes, supporting circCalp as a sensitive biomarker of transient inflammatory response to diving-related physiological stress.

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L-06

Effect of repeated recreational SCUBA diving on the pituitary-target organ axis

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Introduction: Recreational self-contained underwater breathing apparatus (rSCUBA) diving is a moderate-intensity physical activity performed under unique environmental conditions that affect hormonal regulation and homeostasis. Hormonal responses during diving primarily involve activation of the sympathetic-adrenal-medullary and hypothalamic-pituitary axes. This study aimed to investigate the effects of repeated rSCUBA diving on the endocrine system by monitoring hormones related to the pituitary-target organ axis, including adrenocorticotrophic hormone (ACTH)-cortisol, thyroid-stimulating hormone (TSH) – free thyroxine (fT4), prolactin-testosterone, and growth hormone (GH) – insulin-like growth factor 1 (IGF-1).

Materials and methods: The study included 14 male recreational divers with a median age of 42 years (range 19-54 years). Participants performed five dives over five consecutive weeks, once weekly between 9 and 10 AM, at depths of 20-30 meters, with each dive lasting 30 minutes. Blood samples were collected immediately before and after the first, third, and fifth dive. Plasma ACTH concentrations, as well as serum GH and IGF-1 concentrations, were analyzed using the Roche Cobas e411 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). Serum TSH, fT4, prolactin, testosterone, and cortisol concentrations were measured using the Abbott Architect i2000SR analyzer (Abbott Diagnostics, Abbott Park, Illinois, United States). Statistical analysis was performed using IBM SPSS Statistics. Differences between pre- and post-dive measurements were evaluated using the Wilcoxon signed-rank test. P-value < 0.05 was considered statistically significant.

Results: A statistically significant decrease in post-dive ACTH concentrations after the first, third, and fifth dives was observed ($P = 0.001$, $P = 0.004$, and $P = 0.002$, respectively), accompanied by a statistically significant decrease in cortisol concentrations ($P = 0.006$, $P = 0.019$, and $P = 0.022$). Although testosterone levels were lower after each dive, a statistically significant difference was observed only after the first dive ($P < 0.001$). No significant changes were observed for other measured hormones.

Conclusion: Repeated weekly rSCUBA diving does not induce physiological stress in experienced divers and promotes enhanced parasympathetic nervous system activity, as indicated by a reduction in ACTH and cortisol concentrations after each dive.

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M New biomarkers

M-01

TGF- β 1 concentrations in exosomes obtained by liquid biopsy from the plasma of patients with colorectal adenoma and colorectal cancer

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Introduction: Liquid biopsy was developed as a non-invasive approach for analysing tumour-associated elements, including exosomes, from peripheral blood. We hypothesized that exosomes carrying tumour-derived molecules, such as transforming growth factor- β 1 (TGF- β 1), differ in their content depending on colorectal cancer (CRC) stages. Changes in the TGF- β 1 signalling pathway lead to uncontrolled cell growth, tumour progression, and metastases. Therefore, this study aimed to examine differences in TGF- β 1 concentrations in exosomes from the plasma of patients with colorectal adenoma (CRA) and CRC.

Materials and methods: Exosomes were isolated from the plasma of patients with CRA (N = 24) and CRC (N = 40) using the miRCURY Exosome Serum/Plasma Kit (Qiagen, Germany). Protein concentration was determined by the spectrophotometric method with bicinchoninic acid and CuSO₄ (Sigma-Aldrich, USA). Western blot analysis was performed using a mouse monoclonal antibody to detect TGF- β 1 (Invitrogen, USA) in exosomes, with relative quantification of TGF- β 1 protein bands. Statistical analysis was performed using the Mann-Whitney test in the MedCalc program (MedCalc Software Ltd, Ostend, Belgium).

Results: There were no differences between CRA and CRC patients in the relative intensities of exosomal TGF- β 1 protein bands at approximately 135 kDa (which represent a latent form), with $P = 0.524$. However, the sum of the relative intensities of the TGF- β 1 protein bands at approximately 44 kDa, 25 kDa and 12 kDa (that are considered active forms), as well as the ratio of the active forms and the latent form, were statistically significantly higher in the exosomes of CRC patients as compared to those of CRA patients ($P < 0.001$ and $P = 0.011$, respectively).

Conclusion: Different TGF- β 1 concentrations present in exosomes of CRA and CRC patients indicate a potential of liquid biopsy as a non-invasive approach for monitoring tumour progression and the development of metastases.

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M-02 (Oral presentation)**The association of concentration of urine calprotectin with sterile leukocyturia in patients with bladder cancer**

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Introduction: Bladder cancer is the most common malignancy of the urinary system. Urinary calprotectin, the heterodimer of S100A8/S100A9, has been investigated as a potential biomarker in bladder cancer. Our aim was to investigate the association between sterile leukocyturia and urinary calprotectin in patients with bladder cancer.

Methods: Cross-sectional study that included spot urine samples of 48 patients with bladder cancer and sterile urine culture undergoing transurethral resection of bladder at the Department of Urology, University Hospital Sveti Duh was performed in the first half of 2025. The diagnosis of bladder cancer was confirmed by histology analysis of tissue samples. Urinary calprotectin was analysed using automated *in vitro* diagnostic test for the quantitative determination of calprotectin in human stool specimens (Bühlmann fCAL turbo, Bühlmann Laboratories AG, Schönenbuch, Switzerland), with the dilution factor of 500 adjusted for measurements in urine on Atellica Solution automated biochemistry analyzer (Siemens Healthineers, Erlangen, Germany). Urine analysis was performed using Siemens Clinitek Novus automated analyzer (Siemens Healthineers, Erlangen, Germany). Definition of sterile leukocyturia was > 15 leukocytes in μL of urine with sterile urine culture. Data (median and interquartile range) was analyzed using Mann-Whitney test and Spearman's rank correlation test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using MedCalc Software v12.5.0.0.

Results: Median urinary calprotectin in patients with sterile leukocyturia was significantly higher than in patients without leukocyturia (1.12 mg/L (0.18-2.65 mg/L) vs. 0.15 mg/L (0.06-0.49 mg/L); $P = 0.006$). Spearman correlation revealed a significant correlation between urinary calprotectin and leukocytes counts ($\rho = 0.551$, $P < 0.001$).

Conclusion: There is an association between urinary calprotectin concentration and sterile leukocyturia. Therefore, the specificity of urinary calprotectin as a biomarker of bladder cancer in the presence of sterile leukocyturia should be thoroughly examined in a larger study.

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M-03 (Oral presentation)**Serum GFAP and UCH-L1 in relation to CT findings in traumatic brain injury**

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Introduction: Traumatic brain injury (TBI) is defined as a disruption of brain function by external forces to the head. Glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase-L1 (UCH-L1) are blood biomarkers that are able to aid in the assessment of mild TBI (mTBI) patients and reduce computed tomography (CT) overuse. The aim of this study was to evaluate the association of serum GFAP and UCH-L1 with CT findings in patients with mTBI.

Materials and methods: Suspected TBI patients admitted to the UCH Mostar Emergency Department were enrolled in the study. Clinical evaluation included the Glasgow Coma Scale (GCS) assessment and a head CT scan. Serum GFAP and UCH-L1 were measured by CMIA on the Alinity ci analyzer (Abbott). Positive biomarker status was defined as GFAP ≥ 35.0 ng/L and/or UCH-L1 ≥ 400.0 ng/L. Based on the combination of biomarker concentration and CT findings, patients were categorized into three groups: Group 1 (negative biomarkers/negative CT, N = 26), Group 2 (positive biomarker(s)/negative CT, N = 64), and Group 3 (positive biomarker(s)/positive CT, N = 47). Data analysis was performed using MedCalc (v. 22.006, Ostend, Belgium).

Results: The median GFAP concentration in Group 1 was 16.1 (12.6-24.6) ng/L while in Groups 2 and 3, the values were 89.1 (40.7-150.6) ng/L and 331.6 (157.9-692.2) ng/L. UCH-L1 concentrations showed a median of 155.2 (110.6-259.9) ng/L in Group 1, compared to 577.6 (354.6-1019.6) ng/L and 417.5 (226.1-868.9) ng/L in Groups 2 and 3. Statistically significant differences in GFAP concentrations were observed between all three groups ($P < 0.001$), indicating that this biomarker effectively discriminates between each group ($P < 0.05$). UCH-L1 concentrations were significantly higher in Groups 2 and 3 compared with Group 1 ($P < 0.001$), although no significant difference was found between them ($P > 0.05$).

Conclusion: GFAP shows strong association with CT outcomes and discriminates all groups, whereas UCH-L1 differentiates only CT-negative from CT-positive patients.

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M-04

Comparative performance of the ADAPT algorithm and FIB-4 index in a prospective cohort: the diagnostic value of PRO-C3 in identifying liver cirrhosis

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Introduction: Accurate assessment of liver fibrosis is essential for managing chronic liver diseases. While the FIB-4 index is a widely used screening tool, its specificity is often limited as it does not directly reflect active tissue remodeling. N-terminal pro-peptide of type III collagen (PRO-C3) serves as a direct biomarker of fibrogenesis. This prospective study evaluated the diagnostic performance of the ADAPT algorithm (PRO-C3, age, diabetes status, and platelet count) compared to the FIB-4 index, with validation against clinical and ultrasound findings.

Methods: Patients were prospectively enrolled for a comprehensive evaluation, including physical examination, abdominal ultrasound for morphological assessment and serological profiling. PRO-C3 levels were measured using the cobas pro analyzer (Roche Diagnostics, Basel, Switzerland). ADAPT and FIB-4 scores were calculated simultaneously. Statistical analysis utilized Pearson's correlation to assess associations with advanced fibrosis, while t-tests compared the discriminative power between early-stage (F0/F1) and cirrhotic (F4) patients.

Results: A high diagnostic concordance was observed between laboratory algorithms and clinical morphology. The ADAPT algorithm exhibited a superior correlation with advanced fibrosis ($r = 0.852$, $P < 0.001$) compared to the FIB-4 index ($r = 0.694$). Mean PRO-C3 levels were significantly higher in the F4 group than in the F0/F1 group. Notably, the ADAPT algorithm provided a more stable and linear reflection of disease progression, aligning precisely with ultrasound findings of architectural distortion and portal hypertension, whereas FIB-4 demonstrated higher variability, particularly in older patients.

Conclusion: This prospective evaluation confirms that the ADAPT algorithm is significantly more accurate than the FIB-4 for identifying advanced liver fibrosis. The strong agreement between the ADAPT algorithm, ultrasound findings, and clinical presentation suggests that this integrated approach can serve as a robust, non-invasive diagnostic tool. Utilizing the ADAPT algorithm enhances diagnostic precision and effectively reduces the necessity for invasive liver biopsies in routine hepatological practice.

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M-05

Plasma levels of inactive matrix Gla protein in patients with colorectal cancer

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Introduction: Matrix Gla protein (MGP) is currently considered one of the most potent inhibitors of tissue calcification. In addition, previous studies have indicated the probable involvement of MGP in carcinogenesis. Increased gene expression of MGP has been observed in various types of tumors, including colorectal cancer. The aim of this study was to compare plasma levels of inactive dephosphorylated and uncarboxylated MGP (dp-ucMGP) in patients with colorectal cancer with levels in control subjects.

Materials and methods: Plasma dp-ucMGP levels were measured in 10 male patients with colorectal cancer treated at the University Hospital of Split, Split, Croatia. The results were compared with levels in healthy male subjects of approximately the same age collected as part of a previously published thesis (Vilović, 2019). EDTA plasma concentrations in both study groups were measured using a chemiluminescent immunoassay (CLIA) performed on the IDS-iSYS analyzer with the IDS-iSYS InaKtif MGP reagent kit (Immunodiagnostic Systems, Liège, Belgium). Statistical analyses were performed using the Mann–Whitney test in MedCalc Statistical Software (MedCalc Software Ltd, Ostend, Belgium).

Results: Plasma levels of dp-ucMGP were significantly higher in patients with colorectal cancer compared to healthy controls (median 649.50 pmol/L, interquartile range (IQR) 591.00-754.00 pmol/L vs median 480.50 pmol/L, IQR 456.00-504.00 pmol/L, $P < 0.001$).

Conclusion: The results of this study encourage further consideration of MGP as a potential biomarker in colorectal cancer.

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N New technologies

N-01 (Oral presentation)

Implementation of the clinical decision support algorithm for screening of hemoglobinopathies: preliminary results from the University Hospital Centre Zagreb

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Introduction: Clinical decision support (CDS) systems are rule-based solutions that are being increasingly implemented within hospital and laboratory information systems (LIS) and can provide added value to the laboratory report by evaluating multiple parameters according to predefined rules. The aim of the present study was to implement the algorithm for screening of hemoglobinopathies within the AlinIQ CDS (Abbott Laboratories, USA) and assess its effectiveness.

Materials and methods: Two-way communication between AlinIQ CDS and LIS (BioNET LIS, IN2, Croatia) was established. AlinIQ CDS evaluates patients' laboratory results continuously and, according to the predefined algorithm, triggers an appropriate comment. Algorithm for screening of hemoglobinopathies includes the following criteria: age between 3 and 60 years, elevated erythrocyte count or RDW, MCV < 72 fL, MCH < 27 pg and hematology wards excluded. If satisfied, the system triggers a comment recommending hemoglobin (Hb) electrophoresis.

Results: In a 5-month period (November 2025-March 2026) for a total of 92 patients a comment was triggered. For all patients, clinical and other laboratory data were inspected, in order to identify those eligible for Hb electrophoresis. Due to other causes of predefined alterations of laboratory results 63 patients were excluded, for 21 patients Hb electrophoresis was already requested while for eight patients Hb electrophoresis was considered relevant and was subsequently performed. In 4/8 patients hemoglobinopathy was confirmed, as follows: hereditary persistence of fetal Hb in two cases (29.9% and 15.4%, respectively), one case of Hb Lepore syndrome (10.8%) and one beta-thalassemia minor case (HbA2 = 5.9%).

Conclusion: The implemented approach that combines automated screening of routine patients according to predefined rules within AlinIQ CDS and subsequent cognitive decision on further diagnostics made by laboratory specialists was proven to be effective for proper identification and eventually diagnosis of unrecognized hemoglobinopathies. When designed carefully, CDS systems enhance the use of laboratory data and improve patient care.

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N-02

Are you made of stone? - Establishment of indirect reference intervals for urinary oxalate and citrate

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Introduction: Establishing direct reference intervals (RI) imposes significant time and financial burdens due to healthy control selection and analytical costs. In the setting of urinary biomarkers, this is further complicated by adequate 24-hour urine collection. The aim of the present study was to calculate indirect reference intervals for urinary oxalate and citrate determined by ion chromatography.

Materials and methods: From June 2022 to October 2025, 1243 unique urinary oxalate (782 female) and 1172 citrate results (730 female) from an adult population were extracted from the BioNET LIS (IN2, Zagreb, Croatia). Indirect RIs were estimated using the RefineR (v2.0.0) package in R (v4.5.3). To determine 95% confidence intervals (CI) for the lower (LL) and upper limits (UL), 200 bootstrap iterations were performed. Percentage agreement with routinely used RIs (15–32 mmol/mol creatinine for oxalate; 107–653 mmol/mol creatinine for citrate) was validated using 40 independent patient samples with identical preanalytical requirements.

Results: Calculated indirect RIs were 6.9 (CI: 4.6–7.9) - 42.9 (CI: 35.7–51.2) mmol/mol creatinine for oxalate and 17 (CI: 15–68) - 449 (CI: 360–542) mmol/mol creatinine for citrate. Oxalate parameters ($\lambda = 0.376$, $\mu = 5.5$, $\sigma = 1.4$, $NP = 0.939$) indicated successful transformation of asymmetric data, while citrate parameters ($\lambda = 1.000$, $\mu = 180$, $\sigma = 134$, $NP = 0.924$) suggested a normal distribution. In 40 test samples, the current RI showed 11 outliers for oxalate and 6 for citrate. Conversely, the new indirect RI yielded only one oxalate and two citrate outliers, demonstrating superior representation of the adult population.

Conclusion: Indirect reference interval assessment from large datasets enables more accurate local population modeling. This new RI reduces false-positive hyperoxaluria and hypocitraturia, minimizing unnecessary clinical burdens, treatment costs, and patient stress.

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N-03 (Oral presentation)**Clinical validation of digital PCR-based ESR1 mutation detection in circulating cell-free tumor DNA from patients with breast cancer**

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Introduction: Estrogen-receptor alpha gene (ESR1) mutations are clinically relevant somatic alterations in metastatic estrogen receptor-positive (ER+) breast cancer (BC) and are associated with acquired resistance to endocrine therapy. Due to marked tumor heterogeneity in advanced disease, tissue biopsy may not capture emerging resistant subclones. Liquid biopsy enables analysis of circulating cell-free tumor DNA (ctDNA) from primary and metastatic lesions, but highly sensitive methods are required due to low ctDNA abundance. The aim was to validate digital PCR-based ESR1 mutation detection at very low input ctDNA concentrations and clinically evaluate the assay in patients with BC.

Materials and methods: Circulating cell-free tumor DNA was isolated from K-EDTA plasma using the QIAamp Circulating Nucleic Acid Kit on the QIAvac 24 Plus system (QIAGEN, Germany). ESR1 mutation analysis was performed using the ESR1 17 mutations Crystal Digital PCR Assay on the NioE digital PCR system (Stilla Technologies, France). The assay detects 16 ESR1 mutations across exons 7 and 8. Analytical validation of the lowest input ctDNA concentration was performed by serial dilution from 5 ng/μL–0.039 ng/μL using ctDNA from one patient harboring ESR1 p.(Asp538Gly) and a positive control containing all target mutations. Clinical validation included 31 patients with BC, including 25 with metastatic ER+ disease. Results were analyzed descriptively.

Results: Reliable absolute quantification of at least 1 copy/μL of mutated DNA was achieved at 0.039 ng/μL ctDNA input. Among 25 patients with metastatic ER+ BC, ESR1 p.(Asp538Gly) was detected in one patient with endocrine resistance in the sixth line of treatment, at 21 copies/μL. No ESR1 mutations were detected in ER-negative patients.

Conclusion: Digital PCR enables highly sensitive ESR1 mutation detection from very low ctDNA input and supports liquid biopsy for assessing molecular heterogeneity in metastatic BC. Absence of ESR1 mutations in ER-negative patients supports clinical specificity, although larger cohorts are needed.

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N-04

Simultaneous determination of circulating tumour DNA and circulating tumour cells in colorectal cancer

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Introduction: Liquid biopsy has been extensively studied in colorectal cancer (CRC). Due to their diagnostic, prognostic, and predictive value, circulating cell-free DNA (ccfDNA) and circulating tumour cells (CTCs) are intensively investigated, but there is still a lack of data regarding the correlation of these two potential biomarkers with tumour markers, which is the aim of this study.

Materials and methods: This study included 31 patients with CRC enrolled in the HRZZ-IP-2019-04-4624 project. ccfDNA was isolated using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany). ccfDNA concentrations were determined using the Qubit dsDNA HS Assay Kit (Invitrogen, USA) on the fluorometer DS-11 FX (DeNovix, USA). CTCs were isolated from 3 mL of whole blood using the ScreenCell Cyto kit system (Sarcelles, France). Detection and characterization of CTCs were performed by immunofluorescence analysis for cytokeratin and CD45, followed by nuclear DNA staining using 4',6-diamidino-2-phenylindole. Tumour grade was determined in all patients, and serum concentrations of CEA, CA 19-9, and CRP were measured. Statistical analysis was performed using the Spearman rank test (MedCalc Software Ltd, v20.305, Ostend, Belgium), with $P < 0.05$ considered statistically significant.

Results: The following study results were obtained: number of CTCs = 1/3 mL blood (0-2); ccfDNA = 13.77 ng/mL (10.32-19.98); CEA = 3.50 µg/L (2.34-6.58); CA 19-9 = 8.06 kIU/L (6.60-15.42); CRP = 4.0 mg/L (2.1-15.7). All patients had low-grade tumours, while tumour size ranged from 2-6.5 cm. The results did not demonstrate statistically significant correlations between ccfDNA concentration and CTC count, nor with the concentrations of CEA, CA 19-9, and CRP. However, a tendency toward increased ccfDNA concentration and higher CTC counts was observed in patients with larger tumours.

Conclusion: Although our results lacked statistical significance and the study was conducted on a relatively small number of participants, the concentration of ccfDNA and the number of CTCs may represent potential biomarkers in patients with larger tumours.

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O Case report

O-01 (Oral presentation)

Bence Jones protein in serum detected using immunosubtraction electrophoresis

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Introduction: Confirming methods for M-protein presence in serum, a key diagnostic marker for plasma cell disorders, are immunofixation electrophoresis (IFE) and immunosubtraction electrophoresis (ISE). Three steps are involved in both methods: serum protein electrophoresis, immunoprecipitation, and visualization. While IFE results in fixed immunoglobulin molecules on an agarose gel, ISE results in a serum protein electropherogram without immunoprecipitated immunoglobulin molecules.

Materials and methods: A 67-year-old woman was requested to have laboratory tests with suspicion of monoclonal gammopathy. All electrophoretic separations are performed using Sebia systems (Capillary3Octa and Hydrasys2Scan) and materials (Lisses, France). Immunoglobulins are measured on the Alinity c Abbott analyzer (Wiesbaden, Germany), and free light chains (FLC) using Siemens reagents on the Atellica Nephelometer (Erlangen, Germany).

Results: In serum protein migration pattern (SPE), an abnormality in the gamma fraction is detected, suspected to be due to M-protein presence. Immunoglobulin concentrations were IgG 6.01 g/L, IgM 1.21 g/L, and IgA 0.25 g/L. In the ISE electropherogram there was no evidence of reaction with any anti-heavy chain isotype, only with the lambda light chain. Suspected monoclonal light chains (Bence Jones protein) of the lambda type in the ISE migration pattern were confirmed with IFE using antisera specific to FLC kappa and lambda. Furthermore, FLC concentrations were 40 mg/L for kappa and 4790 mg/L for lambda.

Conclusion: Due to differences in principles, ISE is a less sensitive method than IFE for M-protein detection. Therefore, IFE is still the gold standard procedure primarily in follow-up conditions involving the presence of intact M-protein and in the diagnosis of Bence Jones protein. Monoclonal light chains are rarely presented as a peak in SPE; a more common characteristic is hypogammaglobulinemia. In case a suspected peak is visible in SPE, Bence Jones protein is possible to be detected using ISE. Still, this result should be confirmed using light chain IFE.

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O-02

Timely recognized and successfully treated acquired von Willebrand syndrome secondary to myeloproliferative disorder: a case report

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Introduction: Acquired von Willebrand syndrome (AvWS) is a rare acquired bleeding disorder, associated with other diseases, including myeloproliferative disorders (MPD). Clinical and laboratory findings of AvWS are similar to those of hereditary von Willebrand disease, including bleeding from mucocutaneous surfaces. However, since most AvWS patients don't bleed in absence of major trauma/surgery, timely recognition is usually underestimated. The aim was to present a case of the patient diagnosed with MPD in whom early recognition of AvWS and adequate therapy resulted with rapid improvement in laboratory and clinical signs of the underlying disorder.

Materials and methods: A 71-year-old female patient was referred by a general practitioner to the haematology clinic due to extremely high platelet (Plt) count ($2589 \times 10^9/L$). MPD was diagnosed (erythrocytes $6.9 \times 10^9/L$, Plt = $2561 \times 10^9/L$, JAK2 V617F positive). The patient developed palate bleeding without any antedecent trauma or infections. Cytoreductive therapy with hydroxycarbamide was initiated. In addition to basic coagulation tests, targeted laboratory diagnostics focused on Willebrand factor activity (vWF:Ac) and antigen (vWF:Ag) determinations was requested.

Results: Activated partial thromboplastin time was prolonged (37.5 seconds), prothrombin time activity was normal (85%). vWF:Ac was markedly decreased (26%), while vWF:Ag was 105% (within reference interval (RI)), with low vWF:Ac/Ag ratio of 0.25 indicative for qualitative type 2 AvWS. The fourth day after cytoreductive therapy initiation vWF:Ac and Plt were 30% and $2360 \times 10^9/L$, eighth day vWF:Ac and Plt were 48% and $1768 \times 10^9/L$. The twelfth day vWF:Ac was 94% (within RI), vWF:Ag = 121%, vWF:Ac/Ag ratio = 0.78; Plt = $672 \times 10^9/L$ and there was no more spontaneous bleeding.

Conclusion: In our patient with MPD, AvWS was timely recognized by targeted laboratory diagnostics and initiated cytoreductive therapy resulted in rapid improvement in laboratory and clinical signs of the underlying disorder. This case highlights the importance of timely recognition of AvWS as an aid for ensuring adequate therapy aimed at preventing high-risk bleeds and obtaining long-term remission.

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O-03

When EDTA interference hides thrombocytosis: a case of EDTA-induced pseudothrombocytopenia

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Introduction: EDTA-induced pseudothrombocytopenia is a well-recognized *in vitro* laboratory artifact characterized by platelet aggregation mediated by IgG or IgM antibodies directed against antigenic epitopes of the GPIIb/IIIa glycoprotein complex. These platelet aggregates are frequently misclassified by automated hematology analyzers as single giant platelets or leukocytes, resulting in spuriously low platelet counts. Recognition of pseudothrombocytopenia is essential for the distinguishing from real thrombocytopenia, as failure to identify this phenomenon may lead to unnecessary diagnostic workup and inappropriate clinical management of a patient.

Material and methods: A 52-year-old male patient was admitted to the outpatient department and a complete blood count (CBC) test was requested in our laboratory as a part of routine monitoring during treatment for skin cell carcinoma.

Results: Initial laboratory analysis showed a normal platelet count ($386 \times 10^9/L$) accompanied by a "platelet clumps" flag generated by the hematology analyzer (Sysmex XN-1000, Kobe, Japan). Peripheral blood smear examination confirmed the presence of platelet aggregates. Due to the suspected EDTA-related interference, a repeat blood sample, including a sodium-citrate tube, was collected the following day. Repeated analysis demonstrated elevated platelet counts in both the EDTA sample ($517 \times 10^9/L$) and the sodium-citrate sample ($679 \times 10^9/L$). Platelet aggregates were present in the EDTA peripheral blood smear but were absent in the sodium-citrate sample. Based on these findings, EDTA-induced pseudothrombocytopenia was confirmed, and only platelet results obtained from the sodium-citrate sample were considered clinically relevant and reported.

Conclusion: EDTA-dependent antibodies are typically associated with falsely decreased platelet count due to platelet aggregation. However, in rare cases, they may also be present at normal or even elevated platelet counts. These findings suggest that laboratory and clinical interpretation of EDTA-induced pseudothrombocytopenia may require specific attention and recognition in such uncommon situations, such as samples with platelet count within or even above upper limit of the reference interval.

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O-04

Eosinophilic pleocytosis in cerebrospinal fluid: a diagnostic challenge

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Introduction: Postoperative complications following neurosurgical procedures in the posterior cranial fossa may include cerebrospinal fluid (CSF) leakage, pseudomeningocele and aseptic meningitis. Eosinophilic pleocytosis in CSF is an unusual finding, most often associated with parasitic infections, foreign body reactions, or postoperative meningeal irritation. In neurosurgical patients, a diagnostic challenge lies in differentiating bacterial meningitis from an aseptic inflammatory reaction, particularly in the presence of CSF leakage.

Materials and methods: We present a case of a 21-year-old male patient with postoperative pseudomeningocele and CSF leakage in whom significant eosinophilic pleocytosis was detected in the CSF. In January 2026, he was admitted to the Emergency Department at Osijek University Hospital, Croatia due to elevated body temperature and suspected CSF leakage. The patient had undergone a suboccipital craniectomy with resection of a posterior cranial fossa arachnoid cyst two months prior. A sample of clear, colorless CSF was obtained *via* lumbar puncture via lumbar puncture and analyzed in the emergency laboratory. Cell count was determined using the body fluid mode on Sysmex XN-2000 (Sysmex, Kobe, Japan), with cell differentiation confirmation performed manually using microscopy. Biochemical analysis was performed by spectrophotometrically methods using Olympus AU680 analyzer (Beckman Coulter, Brea, USA).

Results: The CSF results showed a pleocytosis with leukocyte count of $181 \times 10^6/L$ (reference interval (RI): 0–5). Differential leukocyte analysis revealed elevated eosinophils (24%, RI: 0). Biochemical evaluation demonstrated elevated total protein 1.203 g/L (RI: 0.170–0.370) and decreased glucose (2.20 mmol/L, RI: 2.45–3.33). Lactate concentration was within the reference interval (2.20 mmol/L, RI: 1.20–2.20).

Conclusion: Following consultation of medical biochemists with a clinician, postoperative meningitis was suspected, manifested in the CSF by pleocytosis with eosinophilia, proteinorachia, and hypoglycorrhachia. In this patient, CSF eosinophilia likely resulted from postoperative aseptic meningitis rather than infection.

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O-05

Hyponatremia as a source of interference in complete blood count measurements

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Introduction: Automated hematology analyzers provide rapid and reliable complete blood count (CBC) results, but certain conditions may interfere with their accuracy. Hyponatremia is common in psychiatric patients, yet its impact on CBC parameters is often underrecognized. The aim of this report was to describe a case of unexpected CBC alterations associated with hyponatremia and to emphasize the importance of recognizing osmolality-related analytical interference.

Materials and methods: A blood sample from a 68-year-old female patient, received from the psychiatric emergency department, was analyzed using an automated hematology analyzer Sysmex XN-550. The results revealed an elevated mean corpuscular hemoglobin concentration (MCHC, 362 g/L), along with decreases in mean corpuscular volume (MCV, from 95.1 to 92.0 fL) and sodium (from 139 to 129 mmol/L) compared with results obtained three days earlier. After excluding common causes of falsely increased MCHC, including hemolysis, lipemia, cold agglutinins, and spherocytosis, hyponatremia was suspected as the underlying cause of the discrepancy. The sample was diluted (1:5) with CELLPACK DCL and incubated at room temperature for 15 minutes prior to repeat analysis.

Results: Following dilution and repeat analysis, MCHC normalized (341 g/L), while MCV values were consistent with prior measurements (96.2 fL on repeat analysis). This confirmed that the initial abnormalities were caused by analytical interference due to decreased sample osmolality associated with hyponatremia.

Conclusion: Severe hyponatremia can cause transient, osmolality-related analytical interference in CBC measurements, as demonstrated by the observed decrease in MCV and falsely elevated MCHC in this case. These changes are not indicative of true hematological abnormalities but rather reflect the effects of reduced plasma osmolality. Awareness of this interference is essential to avoid misinterpretation of laboratory results. When discordant CBC parameters are detected, particularly in patients with electrolyte imbalance, the potential impact of the electrolyte imbalance itself should be considered.

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O-06

Therapeutic monitoring of antiseizure medications in blood during hemodialysis: a case reportMihovil Horvat^{1,2}, Merica Aralica^{1,2}¹Clinical Department of Laboratory Diagnostics, Clinical Hospital Center Rijeka, Rijeka, Croatia²Department of Clinical Laboratory Diagnostics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Introduction: Antiseizure medications (ASM) are pharmacological agents used to control seizures in patients with epilepsy. In general, therapeutic drug monitoring (TDM) is performed in monitoring patients with comorbidities and/or polytherapy, taking into account patient's medical history and ASM pharmacokinetics and pharmacodynamics. ASM therapy in patients with combined epilepsy and impaired renal function on hemodialysis (HD) requires special attention. Here we present the case of a 49-year-old female with chronic kidney disease (CKD), structural epilepsy, and mental disorder together with arterial hypertension, left-sided nephrectomy, right-sided pyelolithotomy, secondary anemia, and left-sided adnexectomy.

Materials and methods: The patient was initially admitted to Department of Nephrology due to urinary tract infection (UTI), but because of repeated grand mal seizures and unconsciousness, she was moved to Intensive Care Unit in July 2025. Apart from HD, she was treated with the following ASMs (in mg): levetiracetam 2x750 plus 750 after HD, oxcarbazepine 2x300, and lamotrigine 2x100. Her additional therapy included: clonazepam, pantoprazole, furosemide, amlodipine, folic acid, vancomycin, piperacillin/tazobactam, and paracetamol. On several occasions, seizures progressed after HD. Therefore, TDM was requested in blood withdrawn immediately before (S1), 2 hours after (S2), and 4 hours after HD (S3). TDM was performed on the Nexera X2, ultra-high performance liquid chromatography with ultra-violet detector (Shimadzu, Japan) using a commercial kit (Recipe, Germany).

Results: The following drug levels were obtained (S1/S2/S3 in $\mu\text{mol/L}$): oxcarbazepine 51.7/34.4/35.4, levetiracetam 634.1/356.1/153.4, lamotrigine 6.9/4.1. Lamotrigine was not reported in S1 due to analytical interference. The listed ASM therapy was adjusted according to TDM results, which resolved the seizures, but the patient eventually died from UTI complications.

Conclusion: This was a case of a patient with a complex medical history aggravated by CKD and epilepsy, leading to HD that limited ASM efficiency. It proved a need for TDM over empirical therapy in resolving poorly controlled seizures.

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O-07

Determination of coagulation parameters using analyzers of different measurement methods in the case of asparaginase-induced lipemia interferencePetra Andrašić¹, Jasna Leniček Krleža^{1,2}¹Department of Medical Biochemistry and Hematology, Children's Hospital Zagreb, Zagreb, Croatia²University Department of Nursing, Catholic University of Croatia, Zagreb, Croatia

Introduction: Pegylated asparaginase is a modification of L-asparaginase used in treating acute lymphoblastic leukemia (ALL). Besides its therapy advantages, asparaginase has some side effects, including hypertriglyceridemia and hypercholesterolemia. The resulting lipemia in samples can interfere with coagulation photometric tests. This study aimed to present a case in which, due to severe asparaginase-induced lipemia, it was not possible to measure prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, and thrombin time (TT) using multiwavelength photometry, but also to show how an optomechanical clot-detection method can provide accurate results in such a situation.

Materials and methods: A four-year-old male patient is being treated for a diagnosis of ALL. After receiving the second dose of pegylated asparaginase, he developed extreme hypertriglyceridemia and hypercholesterolemia despite dietary correction measures and introduction of omega-3 supplements. A plasma sample for coagulation testing was measured on automated Siemens CS-2500 and semi-automated Siemens BFT II analyzers (Siemens Healthineers, Erlangen, Germany) according to the manufacturer's recommendations. Serum tests were analyzed on AU680 (Beckman Coulter, Brea, USA).

Results: Laboratory examinations revealed significant elevation of triglyceride (43 mmol/L) and cholesterol (18 mmol/L) concentrations. The serum indices graded lipemia index as abnormal ('ABN'). For parameters PT, APTT, fibrinogen, and TT, the CS-2500 displayed asterisks (****) with no results and the message 'No coagulation.' Afterward, laboratory professionals decided to test those coagulation parameters on BFT II analyzer due to severe visible lipemia. This time, employing the optomechanical clot-identifying technique, we obtained the following results: PT ratio = 0.88, PT INR = 1.06, APTT = 34.9 seconds, TT = 27.2 seconds. At that moment, the fibrinogen method had not yet been verified on the BFT II analyzer.

Conclusion: Although automated coagulation analyzers using simultaneous multi-wavelength analysis technology have many benefits, in cases where severe lipemia interferes, analyzers that combine mechanical and photo-optical detection principles proved to be a better alternative solution.

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O-08

Laboratory-driven resolution of a diagnostic deadlock in visceral leishmaniasis mimicking multiple myeloma: the impact of EuSpLM-led recognition of secondary hemophagocytic lymphohistiocytosis and prompt infectious disease specialist response

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Introduction: Visceral leishmaniasis (VL), a neglected tropical disease with hypoendemic status in Southern Dalmatia, may mimic hematological malignancies. This case describes how laboratory-triggered targeted diagnostic escalation resolved a 42-day clinical deadlock misattributed to suspected multiple myeloma, revealing VL presenting with secondary hemophagocytic lymphohistiocytosis (sHLH).

Materials and methods: A 50-year-old male presented with fever (40 °C), subsequently low-grade fever, weight loss (15 kg), massive splenomegaly (20.2 cm), and pancytopenia (RBC $3.99 \times 10^{12}/L$; WBC 1.7, ANC 0.57, and PLT $81 \times 10^9/L$). Initial workup suggested multiple myeloma based on 22% bone marrow plasma cells, hypergammaglobulinemia (IgG 47 g/L), and elevated beta2-microglobulin (8.8 mg/L). However, negative immunofixation and balanced serum free light chains excluded monoclonality, indicating polyclonal activation.

Results: Clinical worsening prompted self-initiated readmission one month ahead of schedule. Rapid biochemical progression was marked by a ferritin surge from 2,271 to 15,069 ng/mL and an IgG increase to 63 g/L, in the absence of CRAB (hypercalcemia, renal insufficiency, severe anemia, or bone lesions). Profound hyperferritinemia, coupled with splenomegaly, cytopenia, fibrinogen (2.05 g/L), and discordant CRP (22 mg/L), raised strong suspicion of sHLH. A specialist in medical biochemistry and laboratory medicine (EuSpLM) initiated reflex IL-6 testing (57 pg/mL) based on integrated assessment, confirming the hyperinflammatory state and necessitating immediate overnight interdisciplinary communication. EuSpLM-driven targeted bone marrow re-evaluation identified *Leishmania* amastigotes (PCR confirmed). Marked IgG increase reflected infection-induced polyclonal immune activation. Therapy induced rapid clinical remission. Ferritin normalized (216 ng/mL) 7 weeks after treatment initiation.

Conclusion: Visceral leishmaniasis may mimic hematologic malignancy and present with secondary HLH. Recognition of the sHLH signature and polyclonal IgG pattern triggered laboratory-driven diagnostic reassessment, enabling timely diagnosis. Interdisciplinary collaboration is crucial in low-incidence settings. In patients with splenomegaly, pancytopenia, extreme hyperferritinemia, and polyclonal hypergammaglobulinemia, prompt multidisciplinary evaluation with early infectious disease specialist involvement is vital to prevent fatal diagnostic delays.

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O-09

Hidden APL: diagnostic pitfalls and the power of cytogenetics in a life-threatening leukemia

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Introduction: Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia characterized by the PML::RARA fusion gene resulting from t(15;17)(q24;q21). Although it is highly curable with targeted therapy, rapid and accurate diagnosis is essential due to the risk of life-threatening coagulopathy. This case highlights the importance of integrating cytomorphology, immunophenotyping, cytogenetics, and molecular testing in establishing the diagnosis of APL.

Materials and methods: We report the case of a female patient who presented to the emergency department with abnormal blood count findings and multiple hematomas over the preceding three weeks. Initial evaluation, including hematological review, cytomorphology, and immunophenotyping, did not conclusively indicate APL. Conventional cytogenetic analysis showed an additional material on chromosome 17, with suspicion of involvement of 17q. Therefore, a RARA break-apart probe using fluorescence in situ hybridization (FISH) was performed, which demonstrated RARA gene rearrangement. Subsequently, a PML::RARA dual-fusion probe was applied.

Results: Peripheral blood and bone marrow examination revealed leukopenia without definitive morphological features of APL. Coagulation parameters were within reference interval at presentation. Flow cytometry was non-specific and did not clearly indicate promyelocytic differentiation. Cytogenetic analysis suggested structural abnormalities involving chromosome 17q. FISH analysis confirmed RARA gene rearrangement, and subsequent testing verified the presence of the t(15;17)(q24;q21) translocation. Molecular analysis confirmed the PML::RARA fusion transcript, establishing the diagnosis of acute promyelocytic leukemia.

Conclusion: This case demonstrates that APL may be missed by initial morphological and immunophenotypic assessment. Cytogenetic suspicion followed by targeted FISH and molecular confirmation was essential for accurate diagnosis. The case highlights the importance of a rapid, stepwise multimodal diagnostic approach in suspected acute leukemia.

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O-10

Flow cytometry immunophenotyping of cerebrospinal fluid cells in isolated relapse of acute myeloid leukemia in central nervous system - a case report

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Introduction: Acute myeloid leukemia (AML) is primarily manifested by the presence of leukemic myeloblasts in the bone marrow and peripheral blood. Rarely, leukemic cells can infiltrate the central nervous system (CNS). The presence of myeloblasts in the cerebrospinal fluid (CSF) of patients with AML has been documented in 1-4% of cases either at diagnosis, as a complication during treatment or in relapse of the disease. Analysis of CSF cells has an important diagnostic role, especially in cases of isolated relapse of the disease, where myeloblasts are found in the cerebrospinal fluid, while they are absent in the bone marrow. Flow cytometry immunophenotyping (FCI) allows precise identification of leukemic cells in any body fluid, based on the specific expression of surface and cytoplasmic cell markers.

Materials and methods: A 69-year-old man previously diagnosed with AML (FLT3 and NPM1 negative) after allotransplantation of hematopoietic stem cells comes for a regular check-up with a hematology specialist. He complains of dizziness, leg weakness, pressure and neck pain. A complete blood count reveals significant cytopenia. Due to suspicion of AML relapse, a cerebrospinal fluid and bone marrow aspiration were requested, along with cytomorphological and FCI analysis.

Results: FCI analysis of CSF cells showed a large population of cells positive for myeloid cell surface markers CD45, CD33, CD34, CD38, CD117, and partially positive for CD15, CD64 and HLA-DR. This immunophenotype, together with the cytomorphological findings, corresponds to myeloblasts in AML. FCI analysis of bone marrow cells found only 2% of these cells. These results indicate CNS infiltration and no bone marrow infiltration by AML blasts.

Conclusion: Flow cytometry immunophenotyping of cerebrospinal fluid cells is a key diagnostic technique in demonstrating leukemic cell infiltration in the CNS. This method enables rapid identification of malignant cells in CSF with high sensitivity and specificity, even in small delicate samples with low cell count.

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O-11

Resolving spurious hyperinsulinemia in an 83-year-old patient: the crucial role of clinician-laboratory communication

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Introduction: Discrepancies between laboratory findings and clinical presentation often pose a significant diagnostic challenge. In cases of extreme hyperinsulinemia, distinguishing between exogenous administration, insulinoma, and analytical interference is crucial. This case highlights rapid interdisciplinary communication as the most effective tool in identifying preanalytical factors and guiding subsequent laboratory investigations.

Materials and methods: In an 83-year-old female, during a comprehensive laboratory work-up, a critical insulin concentration of $> 21,000$ pmol/L (reference interval (RI): 14-153) was measured using the CMIA method. Recognizing incompatibility with the patient's stable clinical state, the laboratory specialist immediately contacted the referring clinician, who interviewed the patient and confirmed that she had self-administered her routine insulin dose before blood sampling. While this explained the presence of exogenous insulin, the magnitude of the result strongly suggested an additional analytical interference. For confirmation, we further analyzed the sample by measuring the insulin concentration using the CMIA method following polyethylene glycol (PEG) precipitation, while simultaneously testing the untreated sample *via* the ECLIA method. Additionally, C-peptide concentration was also measured in the original sample using the ECLIA method.

Results: The post-PEG insulin concentration was 340 pmol/L, revealing the interference likely due to macroinsulin complexes. The results obtained with the ECLIA method were 393 pmol/L (RI: 18-173) for insulin and < 0.1 nmol/L (0.37-1.47) for C-peptide. These findings were consistent with her history of type 1 diabetes mellitus, confirming that the insulin in her sample was exogenous and supporting the presence of anti-insulin antibodies.

Conclusion: This case was solved because the lab didn't just "report a number" and the physician provided real-time clinical context. It is a perfect example of why the bridge between the clinic and the laboratory is just as important as the technology we use.

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O-12

Severe autoimmune thrombocytopenia with an extreme low platelets: when laboratory diagnostics becomes crucial

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Introduction: Autoimmune thrombocytopenia (immune thrombocytopenia) is characterized by immune-mediated platelet destruction and impaired platelet production. The aim of this report is to present a case of severe thrombocytopenia with an extremely low platelet count and to emphasize the importance of laboratory diagnostics in monitoring disease progression and therapy response.

Materials and methods: A 61-year-old female patient was admitted due to severe thrombocytopenia and spontaneous hematomas involving multiple body regions. Complete blood count was performed using EDTA-anticoagulated blood (BD, Oxford, UK) on a Sysmex XN-2000 hematology analyzer (Sysmex, Kobe, Japan). Platelet count was compared with the reference interval (RI) ($158-424 \times 10^9/L$). Peripheral blood smear was examined using light microscopy to confirm the automated platelet count. Additional diagnostic workup included bone marrow cytology with immunophenotyping and immunological analysis including antinuclear antibodies. Direct anti-platelet antibodies were also determined. Two months before this admission, the patient also had similar laboratory and clinical findings and was treated with corticosteroids.

Results: At admission, platelet count was critically low at $2 \times 10^9/L$ (RI: $158-424 \times 10^9/L$), which was confirmed by peripheral blood smear examination. Other hematological parameters, including hemoglobin (148 g/L, RI: 119-157) and leukocyte count ($7.8 \times 10^9/L$, RI: 3.4-9.7), were within reference ranges. Bone marrow findings were normal, excluding hematological malignancy. Direct anti-platelet antibodies were positive, supporting an autoimmune etiology. The patient previously responded to corticosteroid therapy, with platelet recovery to $165 \times 10^9/L$, followed by relapse after therapy discontinuation.

Conclusion: This case highlights severe autoimmune thrombocytopenia with an extreme low platelet count and relapse after corticosteroid withdrawal. Laboratory findings, particularly platelet count and detection of anti-platelet antibodies, are crucial for diagnosis and monitoring. Continuous follow-up is essential due to the risk of relapse and potentially life-threatening bleeding complications.

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O-13

Endocrine-genetic axis of male infertility: Y chromosome microdeletions and hormonal imbalanceMunevera Zejnilović, Nihad Zejnilović, Sanja Borožan, Dušan Perović, Iman Zejnilović

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Introduction: Male infertility represents a significant clinical and public health concern with a multifactorial etiology, where azoospermia and severe oligozoospermia constitute its most severe forms. The contemporary diagnostic approach relies on the integration of hormonal evaluation and molecular genetic testing, with particular emphasis on the detection of Y chromosome microdeletions, which are identified in approximately 7-15% of infertile men.

Materials and methods: This study presents a case of a 25-year-old male with primary infertility and confirmed azoospermia.

Results: Ultrasound examination revealed reduced testicular volume, while hormonal analysis demonstrated elevated gonadotropins (FSH and LH), along with decreased testosterone and inhibin B levels, consistent with hypergonadotropic hypogonadism. Molecular genetic analysis using polymerase chain reaction (PCR) identified a microdeletion in the AZFa region of the Y chromosome (loss of SY84 and SY86 loci), confirming severe primary testicular insufficiency with nearly complete absence of spermatogenesis and a very poor prognosis for surgical sperm retrieval.

Conclusion: These findings highlight the critical importance of an integrated endocrine-genetic approach in the evaluation of male infertility, not only for accurate etiological classification but also for guiding clinical decision-making, avoiding unnecessary invasive procedures, and enabling timely genetic counseling. Furthermore, emerging evidence suggests an increasing role of epigenetic mechanisms and novel biomarkers in the regulation of spermatogenesis, offering promising perspectives for the development of innovative diagnostic and therapeutic strategies in the future.

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P Pre- and postanalytical phase of laboratory work

P-01

Effect of delayed centrifugation on serum potassium, sodium, and glucose concentrations

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Introduction: Serum tubes are recommended to be centrifuged within 2 h, but delayed centrifugation is a common preanalytical challenge, especially for specimens collected at remote locations. This study evaluated the impact of delayed centrifugation on serum potassium, sodium, and glucose concentrations.

Materials and methods: Blood for routine testing was drawn from 65 patients into two serum tubes with gel separator (Greiner Bio-One, Kremsmünster, Austria). The first tube was centrifuged 30 min after collection, whereas the second tube was centrifuged 3 h after collection (3500 RPM for 10 min). After centrifugation, potassium, sodium, and glucose were measured on the AU480 analyzer (Beckman Coulter, Brea, USA) according to the manufacturer's specifications using proprietary reagents. Differences (%) relative to the first tube were evaluated according to Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) and reference change value (RCV) criteria. RCV was calculated using a two-sided approach ($z = 1.96$, $P < 0.05$) for potassium and sodium, and a one-sided logarithmic approach ($z = 1.64$, $P < 0.05$) for glucose due to the expected unidirectional decrease.

Results: Mean differences (range) were 0.6% (- 6.6 to 9.5%) for potassium, - 0.2% (- 2.1 to 1.5%) for sodium, and - 8.4% (- 18.5 to 1.1%) for glucose. For sodium, none of the 65 comparisons exceeded the CROQALM ($\pm 3\%$) or RCV thresholds ($\pm 2.6\%$). For potassium, 11/65 comparisons exceeded the CROQALM ($\pm 6\%$), and none exceeded the RCV threshold ($\pm 11.1\%$). For glucose, 41/65 comparisons exceeded the CROQALM (- 7%) and 17 exceeded the RCV threshold (- 10.9%), although the mean difference did not exceed the RCV.

Conclusion: Delayed centrifugation up to 3 h had minimal impact on sodium and potassium, while glucose decreased in a substantial number of samples, occasionally reaching levels that could be clinically relevant. These results highlight the importance of timely centrifugation for accurate glucose measurement.

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P-02

Risk analysis of current pediatric eGFR reporting using the Schwartz equation

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Introduction: In our laboratory, the upper reporting limit for pediatric estimated glomerular filtration rate (eGFR) calculated by the Schwartz equation is $> 75 \text{ mL/min/1.73 m}^2$. However, according to the latest KDIGO guidelines, eGFR values $< 90 \text{ mL/min/1.73 m}^2$ may indicate mildly reduced kidney function depending on age and clinical context; in adults, values below this threshold are classified as decreased. Since adolescents often have eGFR values comparable to adults, the current reporting threshold may lead to underrecognition of mildly decreased kidney function. The aim of this study was to determine the proportion of pediatric patients with eGFR values between 75 and $90 \text{ mL/min/1.73 m}^2$ and to evaluate whether eGFR should be reported as a numerical value rather than a descriptive result.

Materials and methods: Data on pediatric eGFR results were extracted from the Laboratory Information System (LIS) for the period from July 2025 to February 2026. eGFR was calculated using the Schwartz equation based on enzymatic creatinine measurements (Alinity c, Abbott) and patient height. The distribution of eGFR values was analyzed, and patients with results between 75 and $90 \text{ mL/min/1.73 m}^2$ were additionally evaluated according to age.

Results: A total of 140 pediatric patients (median age 12 years, range 1-17) had eGFR results during the study period. Thirteen patients (9.3%) had $\text{eGFR} < 75 \text{ mL/min/1.73 m}^2$, 28 patients (20.0%) had $\text{eGFR} 75\text{--}90 \text{ mL/min/1.73 m}^2$ and 99 patients (70.7%) had $\text{eGFR} \geq 90 \text{ mL/min/1.73 m}^2$. All patients with $\text{eGFR} 75\text{--}90 \text{ mL/min/1.73 m}^2$ were older than 10 years, including 16 patients older than 15 years (median age 12, range 10-17).

Conclusion: Nearly one-fifth of pediatric patients had eGFR values between 75 and $90 \text{ mL/min/1.73 m}^2$, which may represent mildly reduced kidney function according to current guidelines, while under the current reporting practice they were considered normal. These findings support reporting eGFR as a numerical value to reduce the risk of underrecognition of mildly impaired renal function.

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P-03 (Oral presentation)**Impact of menstrual cycle phase on CA125, HE4 and ROMA score interpretation in premenopausal women**

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Introduction: Cancer antigen 125 (CA125), human epididymis protein 4 (HE4) and the risk of ovarian malignancy algorithm (ROMA) score are widely used to assess ovarian cancer risk in premenopausal women. Menstrual cycle-related variations may influence their values and affect clinical interpretation. This study aimed to assess differences in these markers across the menstrual cycle and their potential impact on clinical interpretation.

Materials and methods: The study included 27 premenopausal women. CA125 and HE4 were measured on the Roche Cobas Pro e 801 analyzer (Roche Diagnostics, Mannheim, Germany) in serum samples collected in the follicular (days 3 to 5) and luteal (day 21) phases of the menstrual cycle. ROMA score was calculated from measured values. Differences between phases were assessed by the Wilcoxon signed-rank test, with $P < 0.05$ considered statistically significant. Statistical analysis was performed using MedCalc Statistical Software (version 22.005, MedCalc Software Ltd, Ostend, Belgium). Clinical impact was evaluated by determining the number of women reclassified across established cut-off values.

Results: CA125 values were higher in the follicular than in the luteal phase (15.2 [IQR: 10.4-26.8] vs. 11.7 [IQR: 8.7-20.5] kIU/L, $P < 0.001$). No statistically significant differences were observed for HE4 (47.9 [IQR: 41.1-52.0] vs. 48.5 [IQR: 43.6-53.7] pmol/L, $P = 0.239$) or ROMA score (6.7 [IQR: 4.9-8.4] vs. 7.0 [IQR: 5.6-8.4] %, $P = 0.509$). All women with elevated CA125 values in the follicular phase (3/3) were reclassified as normal in the luteal phase. Similarly, 3/5 women with an elevated ROMA score were reclassified as normal.

Conclusion: Menstrual cycle phase significantly affects CA125 values, with higher concentrations observed in the follicular phase, while HE4 and ROMA score do not differ significantly. However, clinically relevant reclassification was observed for both CA125 and ROMA score, suggesting that menstrual cycle phase may lead to misclassification and should be considered when determining sampling timing.

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P-04

Risk analysis: do we need to do microscopic differential blood count examination for every outpatient request?

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Introduction: Risk analysis is an important laboratory tool used to identify problematic issues and optimize processes. It encompasses risk identification, quantification and monitoring. An increased number of manual differential blood count (DBC) requests for outpatients was observed in our laboratory. We aimed to evaluate the potential risk to patient safety if microscopic DBC is not performed for samples with leukocyte count (Leu) and DBC from hematology analyzer within reference intervals (RIs).

Materials and methods: This study included adult (> 20 years) outpatients with requests for complete blood count (Sysmex XN-1000, Kobe, Japan) and microscopic DBC during three-month period. In all samples, both automated and microscopic DBC were performed. Samples with Leu and DBC results within RIs and without flags were considered without indication for microscopic DBC. Thirteen categories of potential errors were defined using five levels of severity (from no harm to potentially life-threatening) and frequency. Risk was analyzed using 5x5 risk matrix, with severity plotted against frequency. A follow-up analysis based on risk analysis was conducted one year later.

Results: Of 998 samples, 550 (55%) had DBC results within RIs by both methods. A total of 399 (40%) results with automated DBC outside RIs were consistent with microscopic results. Risk analysis was performed for 49 (5.0%) samples with pathological changes detected by microscopy but results within RIs obtained by automated DBC. The most frequent error was missed band neutrophils (3.4%), while the most severe errors were missed atypical lymphocytes (0.2%) and metamyelocytes (0.9%). No potentially life-threatening errors were identified. The follow-up analysis demonstrated a reduction of approximately 25% in daily microscopic DBCs.

Conclusion: There is no significant risk to patient safety when microscopic DBC is omitted in adult outpatients with automated DBC within RIs. Microscopic DBC remains essential only for pathological outpatient samples. This approach resulted in a substantial reduction in manual workload and improved laboratory personnel management.

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P-05

Validation of pneumatic tube system for arterial blood gas analysis: a comparison with manual delivery

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Introduction: Pneumatic tube transport systems (PTS) are widely used in modern hospitals to facilitate the rapid delivery of laboratory specimens. However, concerns remain regarding the potential impact of pneumatic tube transport on the integrity of sensitive laboratory samples, especially arterial blood collected in syringes for blood gas analysis. Mechanical forces such as acceleration, vibration, and pressure changes during transport may influence arterial blood gas parameters. Therefore, our aim was to compare manual and PTS transport of arterial blood samples by evaluating their effects on pH, pCO₂, and pO₂ values.

Materials and methods: Arterial blood samples (N = 40) were collected using safePICO syringes and analyzed using ABL90 FLEX PLUS analyzer (both Radiometer, Brønshøj, Denmark). Upon delivery to the laboratory, samples were manually delivered and analyzed. Immediately after first analysis, any residual air was expelled from the syringes, and the samples were re-transported from the same starting point using PTS carriers (Sumetzberger, Vienna, Austria). Agreement between manual and PTS transport was assessed using Bland–Altman analysis and Passing–Bablok regression, with acceptance criteria of 1% for pH and 8% for pCO₂ and pO₂ according to the Croatian Center for Quality Assessment in Laboratory Medicine (CROQALM). Statistical analysis was performed using MedCalc software v11.5.1 (Ostend, Belgium).

Results: Bland–Altman analysis showed that there was no statistically significant constant and proportional difference in pH, pCO₂ and pO₂ between manual and PTS transport. The mean deviations were: pH = 0.0%; pCO₂ = 0.0% and pO₂ = 1.3%. Passing–Bablok analysis confirmed results ($y = -0.01 (-0.12 \text{ to } 0.24) + 1.00 (0.97 \text{ to } 1.02) x$ for pH; $y = 0.03 (-0.16 \text{ to } 0.27) + 1.00 (0.97 \text{ to } 1.02) x$ for pCO₂ and $y = 0.03 (-0.11 \text{ to } 0.07) + 1.01 (1.00 \text{ to } 1.03) x$ for pO₂).

Conclusion: Manual and PTS transport of arterial blood for blood gas analysis yielded comparable results, confirming that sample integrity is preserved during PTS transport.

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P-06

Effect of protease inhibitor use in pediatric urine sample preparation for BDNF and NGF ELISA analysisMerima Čolić¹, Jasna Leniček Krleža¹, Slaven Abdović²¹Department of Laboratory Diagnostics, Children's Hospital Zagreb, Zagreb, Croatia²Department of Pediatric Nephrology, Children's Hospital Zagreb, Zagreb, Croatia

Introduction: Urinary brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are potential biomarkers of pediatric overactive bladder (OAB). Available studies report variable detectability of these neurotrophins in urine samples. Because proteolytic degradation can reduce measurable protein concentrations and thereby affect the detectability of low levels of protein, this study evaluated whether adding protease inhibitors (PI) during urine sample preparation improves the detection of urinary BDNF and NGF.

Materials and methods: Thirty pediatric urine samples were analyzed, 11 from OAB patients and 19 from healthy controls. Midstream urine samples were collected at the time of subjective bladder fullness and processed within 30 minutes of collection. Each sample was divided into two paired aliquots; one was untreated, and the other was treated with cOmplete ULTRA Tablets, Mini, EASYpack Protease Inhibitor Cocktail (Merck, Darmstadt, Germany) *per* manufacturer's instructions. The samples were then centrifuged at 3000xg for 10 minutes at room temperature. Supernatants were stored at - 80 °C until analysis. Urinary BDNF and NGF concentrations were measured using Sigma-Aldrich Human ELISA kits (Merck, Darmstadt, Germany). Paired untreated and treated samples were analyzed on the same ELISA plates. Statistical analysis was performed using MedCalc v22.007 (MedCalc Software Ltd, Ostend, Belgium).

Results: Median age of OAB patients was 8 years (range 4-16), and 13 years (range 8-17) for healthy controls. All urine samples showed neurotrophin concentrations below the lower limits of quantification (BDNF < 0.07 ng/mL, NGF < 6.86 pg/mL). No quantifiable neurotrophin concentrations were thus obtained in either OAB patients or healthy controls, regardless of PI treatment.

Conclusion: The addition of commercially available protease inhibitors during sample preparation did not improve the detectability of urinary BDNF and NGF in pediatric samples. These findings suggest that protease inhibition alone is not enough to overcome the low detectability of urinary neurotrophins with these ELISA methods.

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P-07

Indirect reference intervals for free thyroxine and triiodothyronine on the Beckman Coulter Dxl 9000 analyzer

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Introduction: The use of appropriate reference intervals (RI) is essential for the correct interpretation of thyroid hormone results. The aim of the study was to develop indirect RI for free thyroxine (FT4) and free triiodothyronine (FT3) for the Dxl 9000 analyzer manufactured by Beckman Coulter (Brea, USA).

Materials and methods: The study was conducted at the Clinical Department of Laboratory Diagnostics, Clinical Hospital Center Rijeka. Data from the laboratory information system collected from August 2024 to December 2025 was used. The data was deidentified and refined to exclude patients with no thyrotropin results or result outside the RI, patients with positive thyroid autoantibodies, patients treated in Clinical Department of Nuclear Medicine, Department of Endocrinology, Diabetes and Metabolism Diseases, Clinic for Oncology, Clinic for Anesthesiology, Intensive care and Pain Management and Clinic for Otorhinolaryngology and Head and Neck Surgery. The most recent data from patients with multiple hormone measurements were included in the analysis. Statistical software R, version 4.5.2 was used for the data analysis. The rpart algorithm and the Harris and Boyd method were used to determine the need for division of data into subgroups by sex or age. The refineR algorithm was used for establishment of RI.

Results: Initially, 87,508 results were collected. After exclusion, 33,419 results were analyzed for FT4 (74% females, median age 57, range 0-98) and 22,475 results for FT3 (74% females, median age 58, range 0-98). The analysis of proposed division of data into subgroups according to sex or age showed that there is no need to divide the data, and universal RI were created. RI with corresponding 95% confidence intervals for upper and lower reference limit are: FT4 7.9 (7.8 - 8.1) - 13.6 (12.9 - 14.2) pmol/L, FT3 4.15 (3.98 - 4.18) - 6.2 (6.09 - 6.24) pmol/L.

Conclusion: The suggested RI can be applied in laboratories measuring FT4 and FT3 on Beckman Coulter analyzers after verification.

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P-08

Reducing the upper reporting limit for serum folate: impact on risk, time, cost and laboratory efficiency

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Introduction: Objectives of this study were to evaluate patient risk linked with lowering the reporting limit for serum folate from > 90.6 to > 45.3 nmol/L (upper assay linearity limit) and potential savings in turnaround time (TAT) and costs by eliminating automatic dilution for results exceeding the linearity limit (LL).

Materials and methods: A retrospective analysis was conducted using laboratory information system data for all serum folate measurements from January 2022 to October 2025. Folate concentrations were measured using a chemiluminescent microparticle immunoassay (CMIA) on the Alinity i analyzer (Abbott, Abbott Park, USA). Results were categorized into groups: (a) below upper reference limit (URL; < 34.9 and < 36.1 nmol/L for males and females), (b) between URL and LL (45.3 nmol/L), (c) between LL and reporting limit (RL) after 1:2 dilution (45.3 – 90.6 nmol/L) and (d) above RL (> 90.6 nmol/L). Risk assessment was performed using Risk Priority Number (RPN), calculated as the product of occurrence, severity and detection, with values 0-3, 4-8, and 9-18 defined as low, moderate and high risk. Time and cost savings were estimated based on the number of additional tests required for automatic dilution.

Results: A total of 54,559 results were analyzed. Most (96.4%) were in groups (a) and (b). Results above LL and RL accounted for 2.2% and 1.5% of cases. For most categories, RPN was ≤ 3 (low risk), while moderate risk (RPN = 6) was observed for concentrations between 45.3 and 90.6 nmol/L. Eliminating automatic dilution reduces TAT by 50 min/sample, saving 99,300 min (69 days) and 6000 €.

Conclusion: Lowering the reporting limit to > 45.3 nmol/L and eliminating automatic dilution presents low to moderate patient risk due to the low frequency of high folate concentrations. Improvements in TAT and cost reduction support implementation of this change, with optional dilution retained for specific clinical requests.

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P-09

Can reduced-volume capillaries replace standard ones in routine acid–base analysis?

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Introduction: Capillary blood sampling is a less invasive alternative to arterial sampling for the assessment of acid-base status. The use of reduced-volume capillaries may reduce total blood loss, which is especially important in neonates, children, and patients requiring frequent sampling. This study evaluates the comparability of acid-base analysis results between reduced-volume (70 µL) and standard capillaries (125 µL).

Materials and methods: A total of 69 paired capillary samples were collected in parallel using standard and reduced-volume safeCLINTUBES capillaries (Radiometer, Brønshøj, Denmark). All samples were analyzed immediately on ABL 90 Flex analyzers (Radiometer, Brønshøj, Denmark). Parameters measured included pH, pCO₂, pO₂, K⁺, Na⁺, Ca²⁺, and Cl⁻. Results were compared using Bland-Altman and Passing-Bablok regression analyses, while the clinical significance of observed differences was assessed according to Croatian Center for Quality Assessment in Laboratory Medicine (CROQALM) predefined allowable limits (pH = 1%; pCO₂ = 8%; pO₂ = 9%; K⁺ = 6%; Na⁺ = 4%; Ca²⁺ = 4%; Cl⁻ = 4%). The statistical analysis was conducted using MedCalc v22.023 (MedCalc, Ostend, Belgium).

Results: Bland-Altman demonstrated statistically significant constant and proportional difference for pH (-0.006 (95% CI: -0.011 to -0.001) and -0.081% (95% CI: -0.148 to -0.014), respectively) and pO₂ (-0.272 (95% CI: -0.511 to -0.032) and -2.923% (95% CI: -5.483 to -0.362), respectively), whereas Passing-Bablok showed no statistically significant differences. For pH, the differences were not clinically significant (mean bias 0.08%). For pO₂, although the mean bias (4%) was within acceptable limits, proportional difference exceeded the acceptable limit in more than 5% of pairs, indicating both statistical and clinical significance. For electrolytes and pCO₂ no statistically significant differences were observed.

Conclusion: All measured parameters demonstrated good comparability between standard and reduced-volume capillaries. Observed differences in pO₂ reflect the physiological variability and known limitations of capillary blood for oxygenation assessment rather than a systematic error, and do not affect clinical interpretation. Reduced-volume capillaries are suitable for routine implementation and can be used interchangeably with standard ones.

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P-10

Are current ferritin cut-offs adequate? Insights from indirect reference intervals and ROC analysis

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Introduction: Ferritin is a key marker of iron (Fe) status, but its interpretation is limited by its role as an acute-phase protein and suboptimal manufacturer-provided reference intervals (RIs). This study aimed to derive indirect reference intervals (iRIs) for ferritin and evaluate its diagnostic performance for Fe deficiency.

Materials and methods: A retrospective study was performed using data extracted from the laboratory information system. Only subjects aged ≥ 20 years with CRP < 5 mg/L were included. The dataset covered the period from January 2022 to December 2025 (women N = 9549; men N = 6972). Ferritin concentrations were measured using a chemiluminescent microparticle immunoassay on the Alinity i (Abbott). Subgroups included: (1) no inflammation (CRP < 5 mg/L), (2) CRP < 5 mg/L + Fe below RI (low), and (3) CRP < 5 mg/L + low Fe + anemia (WHO guidelines). iRIs (RefineR) were calculated and compared with manufacturer-provided RIs (4.6 - 204 μ g/L for women and 21.8 - 274.7 μ g/L for men). Receiver operating characteristic (ROC) analysis was performed to assess diagnostic performance. Statistical analysis was performed using MedCalc software v22.006. (Ostend, Belgium).

Results: In subjects without inflammation (CRP < 5 mg/L), iRIs were higher and wider (women: 11 - 394 μ g/L; men: 39 - 758 μ g/L). After stratification for low Fe, intervals narrowed (women: 4 - 60 μ g/L; men: 5 - 222 μ g/L) and were lowest in the subgroup with low Fe and anemia (women: 3 - 29 μ g/L; men: 4 - 123 μ g/L). ROC analysis demonstrated satisfactory diagnostic performance. In men, area under the curve (AUC) was 0.88 (95% CI 0.87 - 0.89), with an optimal cut-off ≤ 79 μ g/L (sensitivity 80%, specificity 81%). In women, AUC was 0.85 (95% CI 0.84 - 0.86), with an optimal cut-off ≤ 33 μ g/L (sensitivity 80%, specificity 77%).

Conclusion: Derived iRIs differ substantially from manufacturer-provided ranges and are strongly influenced by iron status. These findings support the use of stratified, data-driven reference intervals to improve clinical interpretation of ferritin and reduce misclassification of Fe deficiency.

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P-11

Analysis of sample recollection after reporting suppressed results of hemolysis-sensitive tests in Karlovac General Hospital

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Introduction: Practical recommendations for managing hemolyzed samples recommend suppressing results of hemolysis-sensitive tests measured in samples when H-index exceeds the test-specific cut-offs. Suppressing all results and requesting recollection of another sample means subjecting the patient to another invasive procedure, may delay diagnosis and cause another potential harm to the patient, as well as additional costs. The aim of this study was to analyze the number of sample recollection following the report with suppressed results of hemolysis-sensitive tests.

Materials and methods: In a retrospective analysis of data from the Laboratory Information System in the Department of Medical Biochemistry Laboratory, Karlovac General Hospital in a six-month period the number of reports with suppressed results of hemolysis-sensitive tests and the number of recollected samples after the reports with suppressed results was evaluated. The reports were divided into two groups according to H-index (H-index exceeding 0.3 g/L or 0.7 g/L) measured spectrophotometrically on the Alinity ci analyzer (Abbott Diagnostics, Chicago, USA).

Results: The number of reports with suppressed results of hemolysis-sensitive tests was 490, 394 with H-index exceeding 0.3 g/L (80.4%) and 96 with H-index exceeding 0.7 g/L (19.6%). Sample recollection for determination of suppressed results was performed in 43 cases (8.8%). 41 sample recollections were performed for reports where H-index exceeded 0.7 g/L (42.7% of reports where H-index exceeded 0.7 g/L; 8.4% of all reports with suppressed results) and 2 for reports where H-index exceeded 0.3 g/L (0.5% of reports where H-index exceeded 0.3 g/L; 0.4% of all reports with suppressed results).

Conclusion: Sample recollection following the report with suppressed results of hemolysis-sensitive tests was observed in 8.8% of requests. Given the negligible number of sample recollections, it is useful to issue a report with a suppressed results of hemolysis-sensitive tests.

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P-12

Abnormal serum protein electrophoresis in hemodialysis patients

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Introduction: Serum protein electrophoresis (SPE) is used in clinical practice to identify patients with multiple myeloma and other serum protein disorders. In hemodialysis patients, serum is mostly collected from a central venous catheter (CVC) and may contain residual heparin, which can lead to incomplete clotting and, consequently, fibrinogen interference in SPE. This interference is manifested as a peak between the beta and gamma fractions, which may result in misinterpretation of findings and unnecessary additional medical procedures. This study aimed to compare SPE results in hemodialysis patients after sampling in clot activator tubes (CAT) and rapid serum tubes with thrombin (RST) (Becton Dickinson, New Jersey, USA).

Materials and methods: Comparability was assessed in 62 serum samples from hemodialysis patients older than 18 years, analyzed by capillary electrophoresis using the Minicap Flex-Piercing system (SEBIA, France). SPE was performed on samples collected in both tube types from all patients admitted to the Department of Medical Biochemistry over a four-week period in 2026. Statistical analysis was performed using MedCalc for Windows, version 12.4.0.0 (MedCalc Software, Mariakerke, Belgium). The McNemar's test was used for group comparisons.

Results: A total of 62 paired samples (same patient, two collection tubes: CAT vs RST) were analyzed for the presence of a fibrinogen peak on serum protein electrophoresis. Agreement between tubes was observed in 56/62 samples (0.90). Discordant results were found in 6 cases (0.10), all showing a fibrinogen peak in CAT samples but not in RST samples, while no cases showed the opposite pattern. McNemar's test demonstrated a statistically significant difference between tube types ($p = 0.031$). The overall difference in detection rate was -9.68% (95% CI: -17.04 to -2.32), indicating a lower frequency of fibrinogen peak detection in RST tubes.

Conclusion: The type of collection tube significantly influences the detection of fibrinogen peaks on serum protein electrophoresis.

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P-13

Effect of short aerobic activity on routine serum biochemistry parametersNela Puček¹, Robert Kolman², Vesna Šupak Smolčić^{2,3}, Lidija Bilić-Zulle^{2,4}¹Faculty of Medicine, University of Rijeka, Rijeka, Croatia²Clinical Department of Laboratory Diagnostics, Clinical Hospital Centre Rijeka, Rijeka, Croatia³Department of Clinical Laboratory Diagnostics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia⁴Department of Biomedical Informatics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Introduction: The preanalytical phase includes all activities performed before sample collection and it is considered critical since most laboratory errors arise here. This study investigated how short aerobic physical activity affects basic biochemical parameters in serum, under the hypothesis that physical activity may significantly influence laboratory test results.

Materials and methods: A total of 34 healthy participants (4 men and 30 women), median age 22 (18-49) years were included in the study conducted in the Clinical Department of Laboratory Diagnostics, Clinical Hospital Centre Rijeka during April 2026. Routine laboratory parameters were measured in fasting blood samples collected immediately before short physical activity, and again 15 minutes after exercise. Serum glucose, urea, creatinine, sodium, potassium, chloride, calcium, magnesium, lactate, cholesterol, total proteins, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and creatine kinase were analyzed using Beckman Coulter AU5800 (Beckman Coulter, Brea, USA). The difference between parameter values obtained before and after exercise were assessed using paired t-tests or Wilcoxon tests. Clinical significance was expressed using reference change value (RCV). Graphpad Prism was used for statistical computation, and $P < 0.05$ was set as significant.

Results: Statistically significant differences were observed for glucose ($P < 0.050$), creatinine ($P < 0.001$), calcium ($P < 0.010$), magnesium ($P < 0.001$), total proteins ($P < 0.010$), and lactate ($P < 0.001$). Among these, magnesium levels were significantly lower after exercise, whereas the remaining parameters showed a positive mean change. However, clinically relevant change was observed only for lactate with a 110% increase after exercise when compared to RCV of 86%.

Conclusion: These findings show that even short aerobic physical activity is sufficient to affect several routine biochemical serum markers. While some parameters exhibited only statistically significant changes, lactate also demonstrated a clinically significant increase. These results highlight the importance of appropriate control of preanalytical conditions, including recent physical activity, especially when interpreting lactate results.

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P-14

Sample stability of lipoprotein(a) in serum after two repeated freeze-thaw cycles

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Introduction: The manufacturer of the Sentinel lipoprotein (a) (Lp(a)) assay does not provide detailed long-term stability data for frozen samples. Reported stability is only for up to 48 hours at 2-8 °C. The aim of this study was to assess the analytical stability of Lp(a) by comparing concentrations measured in fresh and frozen serum samples after two freeze-thaw cycles.

Materials and methods: Twenty serum samples were analyzed at three time points: T0 (initial sample), T1 (after one month of storage and one freeze-thaw cycle), and T2 (within one month of the first freeze-thaw cycle). Lipoprotein(a) was measured in nmol/L by immunoturbidimetric method on a Beckman Coulter AU5800 platform. Data were analyzed using the Friedman test followed by Wilcoxon signed-rank tests for pairwise comparisons. Bland-Altman analysis was performed to assess agreement between fresh and frozen samples. MedCalc statistical software (version 14.8.1) was used for data analysis. A P value < 0.05 was considered statistically significant.

Results: The Friedman test revealed statistically significant differences between the three time points ($P = 0.000$). *Post-hoc* Wilcoxon tests showed a significant difference between the initial sample (T0) and the first freeze (T1) ($P = 0.002$), as well as between T0 and T2 ($P = 0.001$), while the difference between T1 and T2 was not statistically significant ($P = 0.089$). Bland-Altman analysis showed that Lp(a) concentrations in fresh samples were on average 1.90 nmol/L higher than those measured after the first freeze-thaw cycle. Additionally, no statistically significant difference was observed between the first and second freeze-thaw cycles.

Conclusion: Although the first freeze-thaw cycle resulted in a statistically significant change in measured Lp(a) concentrations, the mean absolute bias of 5.88% remained below the reference range value, indicating that the observed difference is unlikely to be clinically significant.

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P-15

Validation of low-volume lithium heparin blood collection tubes for emergency laboratory testing in intensive care unit patients

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Introduction: Iatrogenic anemia due to phlebotomy-related blood loss is a recognized complication in critically ill patients. Low-volume blood collection tubes help preserve patients' blood volume and reduce the risk of iatrogenic anemia particularly in vulnerable populations such as intensive care unit (ICU) and pediatric patients. We conducted a validation study of the Minicollect Complete low-volume blood collection system to assess its suitability for implementation in routine laboratory practice.

Materials and methods: Paired samples from 30 ICU patients were collected using standard VACUETTE 3 mL and MiniCollect 1 mL lithium heparin tubes (Greiner Bio-One, Austria). After centrifugation (4000 rpm, 10 min), plasma was analyzed on the Alinity c system (Abbott Laboratories, Abbot Park, USA). Measured analytes included bilirubin, liver enzymes, lactate dehydrogenase, creatin kinase, urea, creatinine, electrolytes, CRP, amylase, and lipase. Statistical analysis was performed using MedCalc statistical software (MedCalc Software Ltd, v23.5.2, Ostend, Belgium).

Results: Bland-Altman plot analysis showed constant bias for total bilirubin (95% CI: 0.25 to 0.60), phosphate (95% CI: - 0.02 to - 0.01), potassium (95% CI: - 0.08 to -0.03), chloride (95% CI: - 1.14 to - 0.33) and creatinine (95% CI: - 1.46 to - 0.76) and proportional bias for total bilirubin (95% CI: 0.57 to 4.25), creatine kinase (95% CI: -2.77 to - 0.02), phosphate (95% CI: - 2.59 to - 0.66), potassium (95% CI: - 2.21 to -0.77), chloride (95% CI: - 1.05 to - 0.30) and creatinine (95% CI: - 1.63 to - 0.91). Passing-Bablok regression analysis showed proportional bias for total bilirubin (95% CI: 0.93 to 0.98). None of the biases exceeded predefined acceptable limits so they were not found clinically significant.

Conclusion: Without clinically significant differences between the results obtained using the two blood collection systems, both tube types can be considered acceptable for routine laboratory practice. The use of low-volume blood collection tubes minimizes phlebotomy-related blood loss, thereby contributing to the reduction of iatrogenic anemia risk, particularly in vulnerable patient populations.

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P-16

Evaluation of postanalytical turnaround time in the emergency laboratory: implications for optimization of laboratory workflowDora Rebrek, Ivana Baršić Lapić, Dunja Rogić

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Introduction: Turnaround time (TAT) is a key quality indicator in emergency laboratory medicine. While most studies focus on preanalytical and analytical phases, the postanalytical interval between result validation and clinician review remains insufficiently investigated despite its potential impact on clinical decision-making and patient management. The aim of this study was to evaluate postanalytical TAT in an emergency laboratory by determining the time interval between biochemical result validation and first clinician review within the hospital information system (HIS) and assess the clinical relevance of the currently established target TAT in the emergency laboratory unit „Hitni 2“.

Materials and methods: A retrospective analysis included biochemical samples from hospital ward patients processed in „Hitni 2“. For each sample, time interval between result validation and clinician review of laboratory report in HIS was recorded. Descriptive statistics (mean, median, minimum and maximum review times, interquartile range (IQR)) were calculated. Differences between clinical departments were assessed using Kruskal-Wallis test with $P < 0.05$ considered statistically significant.

Results: The study included 2099 samples. The median review time was 43 minutes (IQR 16 - 90), while the mean was 106 minutes. Statistically significant differences between departments were observed ($P < 0.05$). Dunn *post-hoc* analysis identified significant difference between surgical and routine medical wards, while comparisons involving intensive care units were not statistically significant. Review times ranged from 5 seconds to 116 hours and 42 minutes. Only 62% of results were reviewed within the 60-minute target. Longer review times were associated with higher workload and increased sample volume.

Conclusion: The postanalytical phase contributes significantly to overall TAT in the emergency laboratory medicine and varies across departments. Since a considerable proportion of results are not reviewed within the current TAT target of 60 minutes, reevaluation of the established TAT criteria after clinician consultation may yield a more realistic and clinically relevant quality indicator.

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P-17

Influence of temperature on the stability of lipoprotein(a) in serum samples

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Introduction: Important item of the preanalytical phase of measurement is influence of temperature on stability of analytes in samples during transport from distant sampling locations. According to the declaration of the reagent manufacturer Roche Diagnostics, the stability of lipoprotein(a) in serum samples is 48 hours at 2-8 °C. If samples are not assayed within that time period, samples should be stored frozen at - 70 °C ($\pm$ 10 °C) or below. The aim of this study was to evaluate stability of lipoprotein(a) in serum samples after freezing them at - 20 °C and thawing them after 14 days.

Materials and methods: The study included 21 patients with lipoprotein(a) values from 32 to 329 nmol/L. The 5 mL tubes with serum separator and clot activator (Vacuette, Greiner Bio-One, Austria) were used. Determination of lipoprotein(a) concentration in serum samples was performed on the day of sampling and after 14 days in samples stored at - 20 °C. Measurement method was particle enhanced immunoturbidimetric assay on Cobas c503 system (Roche, Basel, Switzerland). Statistical analysis was made using MedCalc software (MedCalc v23.5.3., Ostend, Belgium) by paired t-test.

Results: There was no statistically significant difference in lipoprotein(a) concentrations ($P > 0.05$) between the values measured immediately after collection and the values measured 14 days after freezing at - 20 °C.

Conclusion: The results show that lipoprotein(a) is stable in serum samples stored at - 20 °C 14 days. It is recommended that each laboratory sets its own stability criteria for individual analytes depending on the requirements of the laboratory's work organization.

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R Biostatistics

R-01 (Oral presentation)

Indirect reference intervals for vitamin D using Refine R

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Introduction: Vitamin D for the Prevention of Disease: An Endocrine Society Clinical Practice Guideline no longer endorses the recommended value of 75 nmol/L as reference cut-off value for measured 25-OH vitamin D in serum. The aim of this study was to calculate vitamin D reference intervals (RI) on local population data using indirect method for alternative result reporting.

Materials and methods: Vitamin D was measured on Beckman Coulter Dxl 9000 (Beckman Coulter, Brea, USA) from August 2024 to October 2025 at the Clinical Department of Laboratory Diagnostics at Clinical Hospital Centre Rijeka. Results were extracted from the laboratory information system, deidentified and cleaned to exclude non-healthy individuals from hospital wards and polyclinic departments. The data were analyzed using R Statistical Software and packages for processing and visualizing data: readxl, dplyr and ggplot2. RI were calculated using the indirect method with the refineR package. Data partitioning was tested using the Harris and Boyd test.

Results: Out of the initial 50,330 results, 38,033 were eligible for RI calculation. Following the new guideline's age stratification, results were split into age groups: ≤ 18 years (N = 1486, 58% female), 19-50 years (N = 11,827, 82% female) and 51-75 years (N = 19,265, 80% female). Calculated RI with 95% confidence intervals for lower and upper reference limit were respectively: 25 (21-32) - 92 (84-110) nmol/L, 24 (23-28) - 98 (94-102) nmol/L and 31 (21-35) - 110 (101-114) nmol/L. Partitioning was not confirmed between the first two groups but was confirmed between the second and third group.

Conclusion: The calculated RIs paint a new picture of population vitamin D values, and provide a new perspective from the previous cutoff value of 75 nmol/L.

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R-02

Prediction of optimal dilution for KFLC measurement in CSF based on albumin and IgG concentrations

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Introduction: Accurate measurement of kappa free light chains (KFLC) in cerebrospinal fluid (CSF) requires appropriate sample dilution to ensure analytical reliability. Incorrect dilution necessitates repeat measurements, increasing reagent consumption, costs, and turnaround time. We hypothesized that CSF albumin and immunoglobulin G (IgG) concentrations could predict the optimal dilution factor. The aim of this study was to develop a model for predicting KFLC dilution.

Materials and methods: This study included CSF samples analyzed for albumin, IgG, and KFLC using a nephelometric system (Atellica NEPH 630, Siemens Healthineers, Germany). KFLC measurements followed pre-defined dilution protocols. A classification decision tree model was developed in JASP (version 0.96.0, University of Amsterdam, The Netherlands) to assign samples to three dilution categories (A, B, or C) based on albumin and IgG values. The dataset was divided into training and validation subsets (80:20), with additional evaluation on a prospective dataset. Model performance was assessed using accuracy.

Results: A total of 589 CSF samples were included. Overall, 47% (278/589) required at least one additional dilution step, including 31% (185/589) requiring one additional dilution (B) and 16% (93/589) requiring further dilution (C). The model stratified samples using an IgG threshold of 53.8 mg/L, with subsequent splits based on IgG and albumin concentrations. Higher IgG combined with lower albumin concentrations was associated with an increased likelihood of additional dilution. Model accuracy was 77% in the validation dataset and 67% in the prospective dataset (N = 36).

Conclusion: The model provides a structured approach for estimating KFLC dilution in CSF samples based on albumin and IgG concentrations. Compared to standard practice, it improves initial dilution selection and reduces repeat measurements. Despite moderate accuracy and lower prospective performance, the model outperforms routine practice and may improve laboratory workflow.

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R-03

Pharmacokinetic modeling of long-acting risperidone: impact of CYP2D6 polymorphismIva Klarica Domjanović¹, Leon Aarons², Lana Ganoci^{3,4}, Mila Lovrić^{5,6,7}, Maja Živković^{8,9}, Nada Božina⁴¹Agency of Medicinal Product and Medical Devices of Croatia, Zagreb, Croatia²Division of Pharmacy and Optometry, University of Manchester, Manchester, United Kingdom of Great Britain and Northern Ireland³Division for Pharmacogenomics and Therapy Individualization, Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia⁴Department of Pharmacology, School of Medicine, University of Zagreb, Zagreb, Croatia⁵Department of Medical Chemistry, Biochemistry and Clinical Chemistry, School of Medicine, University of Zagreb, Zagreb, Croatia⁶Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia⁷Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia⁸Department for Psychiatry and Psychological Medicine, University of Zagreb, Zagreb, Croatia⁹Department of Psychiatry, University of Zagreb School of Medicine, Zagreb, Croatia

Introduction: A population pharmacokinetic model after bi-weekly administration of long-acting injection risperidone was developed to describe risperidone, 9-hydroxyrisperidone and active moiety (AM, sum of risperidone and 9-hydroxyrisperidone) pharmacokinetics; to estimate pharmacokinetic parameter, to evaluate the influence of demographic characteristics and genetic variations (enzyme CYP2D6 and transporters ABCB1 and ABCG2) on risperidone, 9-hydroxyrisperidone and AM pharmacokinetics.

Materials and methods: Plasma concentration data (two blood samples at steady state) of risperidone, 9-hydroxyrisperidone and AM were obtained from 101 adult subjects. Plasma concentrations were measured by HPLC-DAD method. Genotyping of ABCB1 c.1236C>T, c.2677G>T/A, c.3435C>T, ABCG2 c.421C>A and CYP2D6 $\times N^3 \times 4 \times 5 \times 6 \times 10 \times 41$ was performed by TaqMan real-time PCR and long-range PCR; CYP2D6 genotype was translated to metabolic phenotype. Data were analyzed using nonlinear mixed-effect model with the Monolix software package.

Results: The structural model that best described risperidone and AM was a one-compartment first-order absorption model and linear elimination. A joint model was developed for 9-hydroxyrisperidone and was described by a one-compartment first-order absorption model for risperidone and two compartment model with linear elimination model for 9-hydroxyrisperidone. Volume of distribution and absorption rate constant needed to be fixed to the literature values, leaving only clearance to be estimated. Covariates were analyzed against clearance. The covariate that significantly (based on the change in objective function value of at least 3.84 ($P < 0.05$ with one degree of freedom, Chi-Square distribution)) affected the pharmacokinetics of risperidone, 9-hydroxyrisperidone and AM was CYP2D6 polymorphism. Subjects with reduced CYP2D6 activity showed 47% lower risperidone clearance, 32% lower AM clearance, and 43% lower conversion clearance from risperidone to 9-hydroxyrisperidone compared with normal and ultrarapid metabolizers. Goodness of fit plots indicated that the model described the data reasonably well.

Conclusion: Despite having sparse data, the developed population pharmacokinetic models captured the effect of CYP2D6 polymorphism on risperidone metabolism.

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S Laboratory management

S-01

Analysis of nonconformities in the medical biochemistry laboratory of primary health care at the Zagreb county Health Center Velika Gorica: 2021-2023

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Introduction: The quality management system (QMS) or the HRN EN ISO 15189:2022 in Croatian medical biochemistry laboratories (MBL) in primary health care is generally not implemented. The MBL of the Zagreb County Health Center Velika Gorica is not accredited according to this standard but has implemented the QMS. Aim of the study was to analyze nonconformities in laboratory routine work in the period from 2021 to 2023.

Materials and methods: The laboratory has a system for recording nonconformities within the laboratory information system and in forms. Nonconformities are divided into categories: preanalytical (outside the laboratory – OtL, and laboratory – L), analytical, postanalytical and other.

Results: Number of nonconformities by year, 2021, 2022, 2023: 1505, 951 and 1155, respectively (number of patients by year: 54,262, 61,275 and 67,969, respectively). Percentage (%) of nonconformities by year: a) preanalytical: 99.20, 94.95 and 91.86; b) analytical: 0.60, 4.00 and 6.75; c) postanalytical: 0.20, 0.32 and 0.35; d) other (introduced in 2022): not applicable, 0.74 and 1.04, respectively. Percentage of preanalytical errors by year: a) urine sample not submitted/delivered (OtL): 30.27, 45.40, 25.82; b) no referral for testing (OtL): 32.74, 7.10, 3.86; c) sample for testing not delivered – from general practice or nursing homes (OtL): 18.82, 17.50, 15.46; d) hemolyzed sample (L): 9.00, 8.00, 6.03; e) lipemic sample (L): 3.55, 6.50, 2.40; f) coagulated sample (L): 1.00, 0.67, 0.70; g) insufficient parameters entered for LDL-cholesterol calculation (OtL): 2.50, 0.90, 12.06; h) insufficient parameters entered for UIBC calculation (OtL): 11.50, 2.80, 7.82, respectively.

Conclusion: Analysis of our nonconformities shows that the highest number occurs in the preanalytical phase, particularly outside the laboratory. Postanalytical errors are the least frequent. Further analysis of nonconformities, risk assessment, and identification of opportunities for improvement are necessary.

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S-02

Does standardizing pleural fluid analysis improve quality across the total testing process?Lara Milevoj Kopčinović^{1,2}, Adriana Unić¹, Iva Kralj¹, Alen Vrtarić¹, Anita Radman¹, Marijana Miler¹¹Department of Clinical Chemistry, Sestre milosrdnice University Hospital Center, Zagreb, Croatia²School of Medicine, Catholic University of Croatia, Zagreb, Croatia

Introduction: National recommendations for pleural fluid (PF) analysis were implemented in our institution in February 2022. Before implementation, PF analysis was performed using a non-standardized panel; afterwards, a standardized expanded panel was introduced, including paired serum-dependent calculations and structured interpretative reporting. We hypothesized that standardization would improve quality across the total testing process (TTP). This study aimed to compare selected quality indicators (QI) before and after implementation and quantify changes in laboratory performance.

Materials and methods: Selected QI covering the TTP were extracted from the laboratory information system for the pre-implementation (January 2019 - December 2021) and post-implementation period (January 2023 - December 2025). Indicators included the number of patients/samples, number of tests, sample-related nonconformities (e.g. inappropriate container, clotted or unsuitable sample), samples with incomplete or unreported tests (e.g. serum sample not provided), laboratory turnaround time (TAT), and interpretative comments in test reports. Indicators were compared between periods using the chi-squared test, and sigma values were calculated where applicable.

Results: A total of 817 samples from 632 patients were analyzed before, and 906 samples from 735 patients after implementation. The number of individual tests increased from 4716 to 13,414; the median number of tests per sample increased from 6 to 16. Sample-related nonconformities remained unchanged: 18/817 (2.2%; sigma 3.5) before and 20/906 (2.2%; sigma 3.5) after implementation ($P = 0.995$). Samples with incomplete or unreported tests increased from 26/817 (3.2%; sigma 3.4) to 87/906 (9.6%; sigma 2.8) ($P < 0.001$). Median TAT was 43 min before and 52 min after implementation; 74.7% and 63.4% of samples were completed within 1 hour, respectively ($P < 0.001$). Structured interpretative comments increased from none to 95.9%.

Conclusion: The observed increase in incomplete or unreported tests and longer TAT reflects the implementation of an expanded test panel and increased analytical complexity. Overall, standardization maintained sample quality and substantially improved postanalytical reporting through interpretative comments.

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S-03

Risk assessment in the Unit for Electrophoretic and Immunochemical Laboratory Diagnostics, Department of Laboratory Diagnostics, UHC Zagreb

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Introduction: Risk analysis in a medical laboratory is a systematic, proactive process to identify, evaluate, and mitigate potential hazards across the entire laboratory process, ensuring patient safety and result accuracy. Since ISO 15189, in its 2012 version, specified risk management for the first time, this topic came into focus. Aim of this report is to present a risk assessment in the Unit for Electrophoretic and Immunochemical Laboratory Diagnostics, Department of Laboratory Diagnostics, UHC Zagreb.

Materials and methods: Based on the processed and analyzed internal quality assurance and external quality assurance data, recorded non-conformities, and laboratory information system (LIS) data, the following potential failures are identified: methodological weakness, procedural non-conformity, number of service interventions, personnel-related non-conformity, and issuance of an incorrect result. The methodological weakness was assessed using the sigma metric.

Results: Risk assessment of an adverse event occurring as a result of procedural non-conformity, number of service interventions, and personnel-related non-conformities was made with respect to a time interval of one year (2024), while the risk of issuing an incorrect result was assessed based on the number of tests performed in the Unit (LIS data). The impact on patient safety is categorized into one of four descriptive categories: mild, moderate, severe, or critical. None of the assessed risks were categorized as unacceptable. All calculated sigma values were > 3 . The following potential failures were also considered, but for the reasons stated, risk assessments were not made - reagent/calibrator non-compliance, critical result non-compliance, and manual data entry non-compliance - no such non-compliance was recorded or not applicable.

Conclusion: This report emphasizes the importance of locally recorded data: no data, no analysis. Furthermore, the patients can be harmed not only by the issuance of erratic results but also by the failure of certain processes. Good laboratory practice risk management, assessing all laboratory processes, should be carried out to ensure both patients' and personnel's safety.

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S-04

Impact of a document management system on calibration and reagent change recording accuracy in a clinical laboratory

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Introduction: Accurate laboratory documentation is essential for process traceability. We hypothesized that implementation of the digital document management system (Dandelion) would reduce documentation omissions. This study aimed to compare calibration recording completeness before and after Dandelion implementation and to assess whether reagent change frequency was associated with calibration recording omissions in both periods.

Materials and methods: Data were collected during two equivalent periods (1 January - 20 April) in 2025 (paper-based forms) and 2026 (digital recording *via* Dandelion). For 31 biochemistry analytes, the number of calibrations recorded in documentation was compared with the number registered by the analyzer (reference standard). Recorded reagent changes were also compared between periods. The Wilcoxon paired test assessed differences between documented and analyzer-registered calibrations, while Spearman rank correlation evaluated the association between reagent change frequency and calibration omissions. Statistical analysis was performed using MedCalc version 9.4.1.0.

Results: In the paper-based period, 133 calibrations were recorded *versus* 168 registered by the analyzer, resulting in 35 undocumented calibrations (20.8%). The difference was significant ($P < 0.001$), and reagent change frequency correlated with calibration omissions ($r = 0.46$, $P = 0.013$). After Dandelion implementation, 161 calibrations were recorded compared with 177 analyzer-registered, yielding 16 undocumented calibrations (9.0%). Although the discrepancy remained significant ($P = 0.005$), the number of omissions was reduced. Correlation between reagent change frequency and calibration omissions was no longer significant ($r = 0.09$, $P = 0.624$). Reagent changes were recorded more frequently in Dandelion than in paper forms (180 vs. 155, $P = 0.008$).

Conclusion: Implementation of Dandelion reduced calibration documentation omissions, although statistically significant difference indicates room for improvement. The digital system also eliminated the association between reagent change workload and omissions, suggesting greater resilience during high-activity procedures. These findings support digital documentation systems as an effective quality improvement measure in clinical laboratory practice.

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T Method and test validation

T-01

Verification of the cut-off for total prostate-specific antigen in the Croatian population for Snibe MAGLUMI immunoassay platform and method comparison

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Introduction: Prostate-specific antigen (PSA) is an established biomarker used for screening, diagnosis and therapeutic management of prostate cancer. The National Academy of Clinical Biochemistry (NACB) sets the cut-off at 4.0 ug/L for further diagnostic evaluation. MAGLUMI immunoassay analyzers (Snibe Diagnostic, China) are being increasingly introduced in Croatia. The aim of the present study was to verify the applicability of the NACB cut-off for PSA in the Croatian population measured on MAGLUMI immunoassay analyzer series and perform method comparison with the routinely used chemiluminescence immunoassay method.

Materials and methods: For cut-off verification, a total of 90 serum samples from apparently healthy male individuals (median age 38 years; from 18 to 64 years) undergoing regular medical check-up were included. Method comparison was performed using 88 routine samples from male patients admitted for laboratory determination of PSA. Samples were drawn in 3 mL tubes with clot activator (Greiner Bio-One, Austria) and centrifuged for 10 minutes at 2100xg after at least 30 minutes from blood draw. All samples were analyzed on Snibe MAGLUMI X6 analyzer with dedicated reagents, while for method comparison parallel analysis with Alinity i (Abbott Laboratories, USA) was done. Cut-off value was verified following the CLSI EP28-A3c protocol.

Results: PSA values in healthy individuals ranged from 0.07 to 3.34 ug/L. A right-sided cut-off of 2.28 ug/L was obtained, confirming the NACB cut-off of 4.0 ug/L. High comparability between Snibe and Abbott PSA immunoassay was obtained, with Spearman's ρ 0.997 and only small proportional difference obtained by Passing-Bablok regression analysis ($y = 0.00 (-0.02 - 0.02) + 0.95 (0.93 - 0.98) x$).

Conclusion: The universal NACB cut-off of 4.0 ug/L for PSA can be safely used on Snibe MAGLUMI immunoanalyzers in the Croatian population. Excellent comparability between Snibe and Abbott PSA assays indicates high harmonization in the field due to availability of the international reference material for PSA immunoassays.

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T-02

Comparison of lipoprotein(a) measurement on Abbott Alinity and Beckman Coulter DxC 700 analyzers

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Introduction: Lipoprotein(a) is an LDL-like particle recognized as an independent risk factor for atherosclerotic cardiovascular disease. Its plasma concentration is largely genetically determined and remains stable over time, making accurate measurement essential for cardiovascular risk assessment. The objective of this study was to compare lipoprotein(a) results obtained using two routine chemistry analyzers: Abbott Alinity and Beckman Coulter DxC 700.

Materials and methods: Serum samples (N = 14) from adult individuals were analyzed for lipoprotein(a) concentration on the Abbott Alinity (Abbott Diagnostics) and Beckman Coulter DxC 700 AU (Beckman Coulter) analyzers, both employing immunoturbidimetric assays. Statistical analysis was performed using MedCalc Statistical Software version 22.0. The concordance between methods was determined using Passing-Bablok regression and visualized with a scatter plot. The regression equation and correlation coefficient were calculated to assess agreement and potential systematic bias between the two methods.

Results: Lipoprotein(a) concentrations ranged from less than 10 to 520 nmol/L across both analyzers. Passing-Bablok regression analysis yielded the equation: DxC 700 = 1.05 x Alinity + 0.8 nmol/L. The correlation coefficient was 0.996, indicating an excellent linear correlation. Minor discrepancies were observed at low concentration ranges (< 20 nmol/L), while overall agreement remained strong across the analytical range.

Conclusion: The comparison between Abbott Alinity and Beckman Coulter DxC 700 demonstrates excellent correlation and minimal bias in lipoprotein(a) measurement. Both methods can be considered clinically interchangeable, although slight differences may occur at lower concentrations. Continuous standardization and calibration traceability are recommended to ensure consistent lipoprotein(a) testing across different analytical platforms.

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T-03

Comparative evaluation of serum estradiol and progesterone measurement: AIA-CL300 versus Roche Cobas 8000

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Introduction: This study evaluated serum estradiol and progesterone measurements using the AIA-CL300 (Tosoh, Japan) compared with the Roche Cobas 8000 (Roche Diagnostics, Switzerland), aiming to assess the performance and interchangeability of the two platforms.

Materials and methods: Serum samples were collected from patients referred for hormone testing at the University Hospital Center Sestre Milosrdnice, Zagreb, in the Laboratory of Endocrinology. Estradiol and progesterone were measured using Electrochemiluminescence Immunoassay (ECLIA) on Roche Cobas 8000 and Chemiluminescent Enzyme Immunoassay (CLEIA) on AIA-CL300. Method comparison employed Passing-Bablok regression and Bland-Altman analyses. Statistical significance was set at $P < 0.05$ using MedCalc (MedCalc Software, Ostend, Belgium).

Results: For estradiol, Passing-Bablok regression showed a significant systematic bias (intercept 66.47, 95% CI 55.29 - 81.55) and proportional difference (slope 1.17, 95% CI 1.03 - 1.21). Bland-Altman analysis confirmed a significant proportional bias with an arithmetic mean difference of 56.40% (95% CI 43.50 - 69.29%, $P < 0.001$) and an absolute mean difference of 104.38 (95% CI 76.30 - 132.46, $P < 0.001$). In contrast, progesterone showed no significant systematic or proportional bias (intercept - 0.26, slope 1.09; Bland-Altman arithmetic mean difference - 6.89%, $P = 0.1361$; absolute mean difference 8.95, $P = 0.1580$).

Conclusion: Estradiol measurements show significant bias between the two methods, indicating they are not interchangeable and highlighting the importance of using the same platform for patient monitoring. Progesterone measurements are comparable across both methods, supporting reliable clinical use and interchangeability.

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T-04

Verification of reference intervals for the homocysteine enzymatic assay in serum and plasma on the Cobas c 503

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Introduction: Elevated homocysteine is a sensitive marker of vitamin B9 and B12 deficiency and a risk factor for cardiovascular, neurological and metabolic diseases. Reference intervals (RIs) often vary by population, sample type and analytical method, which complicates clinical decision making. This study aimed to compare and verify RIs proposed by the manufacturer (< 12 for fortified vs. <15 µmol/L for non-fortified populations) for the Homocysteine Enzymatic Assay (cobas c 503, Roche Diagnostics). Both serum and plasma primary samples were assessed to determine the most appropriate RIs in adult Croatian population.

Materials and methods: RI verification was performed according to the CLSI EP28-A3 protocol. A total of 42 fasting, apparently healthy adults were initially included based on self-reported health questionnaires. After exclusion of participants with folate or vitamin B12 deficiency, 35 subjects were enrolled (19 men and 16 women) in the study. Simultaneously sampled and processed EDTA plasma and serum samples were stored frozen (-70 °C) until analysis.

Results: The homocysteine concentrations within the manufacturers proposed RIs (< 12 and < 15 µmol/L) was lower in plasma (11/35; 31.4% and 28/35; 80.0%) than in serum (27/35; 77.1% and 35/35; 100%) respectively. Homocysteine concentrations were consistently higher in plasma than serum, as shown by the Bland-Altman mean bias of 19.24% (95% CI: 16.91 - 21.58). Passing-Bablok regression analysis showed no significant proportional (slope = 0.90; 95% CI: 0.73 - 1.07) or constant (intercept = - 1.11; 95% CI: - 3.31 - 1.02) difference. Only the serum RI of < 15 µmol/L met the CLSI acceptance criteria with ≤ 10% of results falling outside the proposed range.

Conclusion: In adult Croatian population, where folic acid fortification is not mandatory, homocysteine should be measured in serum using a RI of < 15 µmol/L. The use of plasma as a primary sample or lower RIs results in an unacceptably high proportion of elevated concentrations lacking clear clinical relevance.

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T-05

Verification of anti-TPO and anti-Tg on MAGLUMI X3 and Abbott Architect i1000SR analyzers

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Introduction: Autoimmune thyroid diseases are associated with antibodies against thyroid proteins, primarily thyroid peroxidase (TPO) and thyroglobulin (Tg), which are important for diagnosis and patient monitoring. This study aimed to evaluate the analytical precision of anti-TPO and anti-Tg measurements using MAGLUMI X3 and Abbott Architect i1000SR analyzers and compare the results with those obtained using the Cobas e 411 as the reference analyzer.

Materials and methods: Repeatability, between-run precision, and within-laboratory precision were assessed using commercial control samples over five days with three replicates *per* run. Coefficients of variation (CVr, CVb, CVI) and bias (N = 15) were calculated and evaluated according to manufacturer specifications and Westgard criteria based on biological variation data. Method comparability with the reference analyzer was evaluated using 30 patient samples *per* parameter. Statistical analysis was performed using MedCalc statistical software version 23.5.2, applying Cohen's κ coefficient to assess agreement in the categorization of results as positive or negative.

Results: Precision results for anti-TPO and anti-Tg on both systems met the criteria defined by Westgard biological variation and manufacturer specifications. Cohen's κ coefficient indicated strong agreement for anti-TPO between the Architect i1000SR and Cobas e 411 analyzers ($\kappa = 0.82$), as well as between the MAGLUMI X3 and Cobas e 411 analyzers ($\kappa = 0.93$). For anti-Tg, moderate agreement was observed between the Architect i1000SR and Cobas e 411 analyzers ($\kappa = 0.78$), and between the MAGLUMI X3 and Cobas e 411 analyzers ($\kappa = 0.79$).

Conclusion: Both the MAGLUMI X3 and Architect i1000SR met predefined precision criteria for anti-TPO and anti-Tg measurements. The analyzers demonstrated good analytical performance and acceptable comparability with the Cobas e 411 analyzer, supporting their use in routine laboratory practice.

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T-06

Verification of the fecal immunochemical test on the OC-Sensor Pledia analyzer

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Introduction: According to the data of the Cancer Registry of the Croatian Institute of Public Health, colorectal cancer is the second most common form of cancer in men (behind lung cancer) and women (behind breast cancer) in Croatia. Since 2007, national colorectal screening program included testing for occult bleeding in the stool using qualitative guaiac fecal blood test. In April 2026, we introduced two OC-Sensor Pledia analyzers (Eiken Chemical, Japan) for quantitative FIT based on the latex turbidimetric immunoassay method to detect hemoglobin concentration. The aim of this study is to verify the performance characteristics of our analyzers by comparing the experimentally calculated values with the manufacturer's declared specifications according to CLSI EP15-A3 guidelines.

Materials and methods: Verification was performed using two levels of commercial control materials (LV1 and LV2). Controls were analyzed in 5 replicates over 5 consecutive days (N = 25) on both instruments. Repeatability and within-laboratory precision were calculated and results compared against manufacturer's declared specification.

Results: For Analyzer 1, the total average concentrations for LV1 and LV2 were 150.0 ng/mL (SD = 1.74 ng/mL; CV = 1.16 %), 451.4 ng/mL (SD = 3.79 ng/mL; CV = 0.84 %). For Analyzer 2, the averages were 148.2 ng/mL (SD = 2.74 ng/mL; CV = 1.85 %), 451.8 ng/mL (SD = 5.29 ng/mL; CV = 1.17 %). Calculated repeatability and within-laboratory precision (CV %) were 1.1%, 1.2% (LV1) and 0.7%, 0.9% (LV2) for Analyzer 1. For Analyzer 2, they were 1.7%, 2.2% (LV1) and 0.6%, 1.2% (LV2). The resulting bias for Analyzer 1 was 0.9% (LV1) and 0.3% (LV2), while for Analyzer 2 it was 0.3% (LV1) and 0.4% (LV2).

Conclusion: The calculated repeatability and within-laboratory precision (CV%) were lower than the manufacturer's declared specifications, with Analyzer 2 meeting the criteria *via* the Upper Verification Limit (UVL) for LV1. The calculated bias for both instruments was within acceptable limits. Both analyzers were successfully verified, demonstrating optimal analytical precision.

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T-07

Analytical performance evaluations of C-reactive protein assay on the Mindray BC-5310 CRP hematology analyzer

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Introduction: Hematology analyzer with C-reactive protein (CRP) option enables simultaneous determination of complete blood count with 5-differential leukocyte formula and CRP from one blood sample. We evaluated the analytical performance of the CRP assay on the Mindray BC-5310 CRP hematology analyzer.

Materials and methods: Repeatability, between run, within-laboratory precision and trueness were determined using the commercial control samples for 5 days in 5 replicates. The coefficients of variation and the bias were calculated (CRP Control L1, L2, N = 25) and compared to the EFLM Analytical Performance Specifications (desirable CVa 17.4% and bias 23.4%). Additionally, concentration of CRP (in EDTA whole blood) on the hematology analyzer Mindray BC-5310 CRP (linearity: 0.2 - 320.0 mg/L) was compared with the concentration of CRP (in the serum) on the Beckman Coulter AU480 chemistry analyzer for 40 patient samples in the range from 1.4 - 273.5 mg/L. The results are statistically processed in the MedCalc program (v.23.5.2) by Passing-Bablok regression.

Results: Satisfactory results were obtained for repeatability, between run and within-laboratory precision; CV at 2.79 mg/L was 1.4%, 1.2%, 1.7% and 1.1%, 0.5%, 1.1% at 36.20 mg/L. Deviation from declared control sample values showed a satisfactory level of accuracy; bias was 3.4% and 1.5%. Concentration of CRP on the hematology analyzer Mindray BC-5310 CRP correlated well with the concentration of CRP on the AU480 chemistry analyzer (the equation of the Passing Bablok and the 95% CI: $Y = 0.06 + 0.99 x$; Intercept A(- 0.29 - 0.40); Slope B (0.96 - 1.02); Cusum test for linearity $P = 0.15$).

Conclusion: We concluded that the CRP assay on the hematology analyzer Mindray BC-5310 CRP is a reliable, precise and accurate method that shows good agreement with the CRP on the Beckman Coulter AU480 chemistry analyzer (there is no deviation between the new and the comparative method, there is no constant or proportional error).

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T-08

The verification of Abbott Alinity c prealbumin method

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Introduction: Prealbumin has a short circulating half-life (≈ 2.5 days) which makes it suitable in assessing short-term nutritional changes in the body. The study's aim was to verify the Abbott Alinity c prealbumin method.

Materials and methods: The verification protocol was conducted on two Abbott Alinity c-series analyzers (A1 and A2) at University Hospital Sveti Duh (KBSD). The verification included imprecision, accuracy and reference intervals (RI). Imprecision was tested according to the CLSI EP15-A3 guideline using two levels of Technopath Multichem S Plus control materials and a pool of leftover serum samples. The coefficients of variations (CV) were compared with the optimal analytical CV from the EFLM Biological Variation Database (EFLM-BV) (2.7%). Accuracy was tested (i) using the commercial Technopath controls according to the CLSI EP15-Ed3-WB; (ii) by comparison of A1 with Alinity c-series analyzer from the University Hospital Center Sestre Milosrdnice (KBCSM) on 40 serum samples stored at -20°C for a maximum of 3 months; and (iii) by comparing A1 and A2 at KBSD on 20 fresh serum samples. Passing Bablok and Bland Altman analysis were used for comparison testing. The EFLM-BV optimal was used as acceptance criterion for accuracy (2.7%). RIs were verified separately for adult men and women on 20 patients per group.

Results: The within-run and between-run CVs for both analyzers were $< 2.7\%$. The observed biases on commercial control materials were 0.280 and 0.920 mg/L for A1 and 3.600 and 6.640 mg/L for A2, namely $< 2.7\%$. A proportional bias of 12.3% was found between KBSD A1 and Alinity analyzer from KBCSM. No clinically significant bias (2.33%) was found when comparing A1 and A2 analyzers at KBSD. All tested samples for men and 18/20 samples for women were within the declared RI.

Conclusion: The Abbott Alinity c prealbumin method demonstrated acceptable analytical performance, indicating it is well-suited for routine clinical use.

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T-09

Verification of manufacturer-reported limits of quantification for TSH and Tg assays using pooled serum samples

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Introduction: Accurate determination of the limit of quantification (LOQ) is essential for the reliability of clinical diagnostic assays for thyroid stimulating hormone (TSH) and thyroglobulin (Tg). The manufacturer-reported LOQs of 0.005 mIU/L for TSH and 0.1 ng/mL for Tg represent the lowest concentrations measurable with an intermediate precision CV of $\leq 20\%$. This study aimed to verify these values using pooled serum samples in accordance with CLSI EP15-A3.

Materials and methods: Pooled serum samples were used as the sample matrix. Five replicates *per* run were performed over five consecutive days (25 measurements *per* sample). For TSH, three pooled serum samples (A, B, C) were prepared, with mean concentrations of 0.008 mIU/L, 0.013 mIU/L, and 0.048 mIU/L, respectively. For thyroglobulin (Tg), two pooled serum samples (A, B) were used with mean concentrations of 0.067 ng/mL and 0.18 ng/mL, respectively. Additionally, a third-party PreciControl Thyrosensitive sample was used with a mean concentration of 0.94 ng/mL.

Results: Repeatability and within-laboratory precision were evaluated and compared against the manufacturer's claims for both analytes. For TSH, repeatability CVs were 6.0%, 5.2%, and 2.4%, with within-laboratory precision CVs of 6.8%, 7.2%, 3.6% for pools A, B, and C, respectively. For Tg, the repeatability CVs were 6.1%, 3.4% and 1.5%, with within laboratory precision of 6.3%, 3.4%, 3.7% for pools A, B, and C, respectively.

Conclusion: All precision estimates fell within the manufacturer's claimed performance goals, confirming that both assays reliably quantify low analyte concentrations with acceptable variability at or near the reported LOQ.

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T-10

Bringing precision to thyroid testing: verification study of the Beckman Coulter Dxl 9000 analyzer from a University Hospital Laboratory in OsijekInja Pavlič¹, Tara Rolić², Nikolina Damašek¹, Marija Vasiljević¹, Ivana Sarić¹¹Clinical Institute of Laboratory Diagnostics, University Hospital Center Osijek, Osijek, Croatia²Faculty of Medicine Osijek, Josip Juraj Strossmayer University, Osijek, Croatia

Introduction: Verification of a new method is essential in clinical laboratories, as differences can significantly influence clinical interpretation, decision-making, and patient management. This is critical in endocrine disorders, where small variations in thyroid hormone measurements may impact on diagnosis and therapy. In 2025, a verification study was conducted according to CLSI EP15 guidelines to evaluate the analytical performance and comparability of two methods: chemiluminescent microparticle immunoassay (CMIA; Abbott, Chicago, USA) and chemiluminescent immunoassay (CLIA; Dxl 9000, Beckman Coulter, Brea, USA). The study was performed at the Institute of Clinical Laboratory Diagnostics, University Hospital Center Osijek, focusing on thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), and free thyroxine (fT4).

Materials and methods: Within-run and between-day precision were assessed using three levels of commercial control material (Lyphochek Immunoassay Plus, Bio-Rad), analyzed in quintuplicate over five consecutive days. Coefficients of variation (CVs) were evaluated against EFLM Biological Variation Database criteria. Method comparison used leftover serums from routine diagnostics. Data were analyzed using MedCalc software.

Results: For TSH, all CVs met optimal performance criteria (CV = 4.5%), with values of 3.1%, 3.1%, and 2.8% for levels L1 - L3. Regression analysis ($y = -0.13 (-0.31 - 0.00) + 1.22 (1.18 - 1.27) x$) showed no significant constant bias; however, CLIA results were on average 14.6% higher than CMIA. For fT3, CVs ranged from 1.8% to 3.1%, meeting minimum to desirable criteria. Regression ($y = 1.48 (0.97 - 2.00) + 0.79 (0.69 - 0.88) x$) indicated proportional bias and lack of agreement. For fT4, CV at L1 (4.0%) didn't meet minimum criteria, while L2 and L3 met desirable specifications. Regression ($y = -1.99 (-3.58 - 0.52) + 1.15 (1.04 - 1.27) x$) demonstrated significant bias, with CLIA yielding higher values.

Conclusion: Acceptable precision was observed for TSH and fT3, while fT4 showed limitations at low concentration levels. Systematic differences between methods were evident. These findings suggest that the evaluated immunoassays may not be fully interchangeable in routine clinical practice.

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T-11 (Oral presentation)

Verification of the reference interval for low dose ristocetin induced platelet aggregation on the Thrombomate XRA analyzerTomislav Hrgović^{1,2,3}, Marija Milić^{2,3}¹Department of Laboratory Diagnostics, National Memorial Hospital Vukovar, Vukovar, Croatia²Institute of Clinical Laboratory Diagnostics, University Hospital Centre Osijek, Osijek, Croatia³Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Introduction: Light transmission aggregometry is considered the gold standard for platelet function testing, employing a recommended initial agonist panel of ADP, arachidonic acid, epinephrine, collagen, and ristocetin. Ristocetin-induced platelet aggregation (RIPA) is essential for diagnosing von Willebrand disease (VWD), particularly subtype 2B, utilizing two different ristocetin concentrations. In healthy individuals, low-dose ristocetin should not trigger significant aggregation. The reference range for most aggregometers is < 15% maximum aggregation (MA). The aim of this study was to verify the reference range for a low ristocetin concentration (0.6 mg/mL) available on the Thrombomate XRA analyzer according to the manufacturer's settings.

Materials and methods: Venous blood was collected from 21 healthy volunteers into 3.2% Na-citrate tubes (BD, Oxford, UK). Platelet-rich plasma (PRP) was prepared by centrifugation at 200xg for 10 min, and platelet-poor plasma (PPP) at 1700xg for 15 min. Platelet aggregation was tested with 0.6 mg/mL ristocetin on the Thrombomate XRA (Behnk Elektronik, Germany) following the manufacturer's protocol. Results were expressed as % MA.

Results: Platelet aggregation exceeded the reference range (> 15% MA) in 14/21 (66.7%) subjects, with values ranging from 16% to 99% MA. Only 7/21 (33.3%) subjects were within the expected range (< 15 % MA).

Conclusion: The verification of the reference range for 0.6 mg/mL RIPA failed, as 66.7% of subjects were above the reference range. According to CLSI EP28 guidelines, no more than 20% of healthy subjects should deviate from the established reference interval. The unsatisfactory verification is highlighted by the clinical inconsistency of the results, as increased RIPA is a hallmark of type 2B VWD, which carries a prevalence of only 0.17% in the general population. Further verification is required using a lower ristocetin concentration (0.5 mg/mL), as recommended by the 2021 British Society for Haematology guidelines.

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T-12

Comparison of follicle stimulating hormone, luteinizing hormone, testosterone and estradiol concentration obtained by Alinity ci and Cobas e402

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Introduction: The results obtained with different immunoassays may differ mostly due to differences in antibody specificity, their reactivity towards different epitopes, calibration standards and detection methods. Laboratories are obliged to perform a method comparison when changing the method or analytical system. The aim of this study was to compare the chemiluminescent microparticle immunoassay (CMIA) with electrochemiluminescence immunoassay (ECLIA) for follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and estradiol determination.

Materials and methods: Methods comparison included analysis of 40 serum samples by both methods, CMIA on Alinity ci (Abbot, Chicago, SAD) and ECLIA on Cobas e402 (Roche, Basel, Switzerland). The obtained results were analyzed using the Bland-Altman and Passing-Bablok regression analysis in the MedCalc program v10.0.2.0 (MedCalc Software Ltd, Ostend, Belgium). The declared analytical sensitivity as well as the reference intervals for all analytes differ.

Results: Passing-Bablok regression indicated proportional and constant difference for comparison of CMIA with ECLIA for FSH ($y = 1.04x - 0.03$), LH ($y = 1.59x + 0.67$), estradiol ($y = 1.09x + 39.91$) as well as for testosterone ($y = 1.85x - 34.5$). Bland-Altman analysis yielded relative biases of 4.4% for FSH (95% CI: 1.94 to 6.82), 41.0% (95% CI: 35.1 to 47.0) for LH, 25.8% (95% CI: 21.3 to 30.4) for estradiol and -26.6% (95% CI: -36.5 to -16.6) for testosterone. The differences between reference intervals provided by the manufacturer are accompanied by obtained differences in relative bias for a particular analyte.

Conclusion: The immunoassays evaluated are not comparable and show significant variability in the results, sensitivity and reference intervals. Observed differences between results of different methods are in this case compensated for by method-specific reference intervals provided by the manufacture. To ensure reliable longitudinal monitoring of patients FSH, LH, testosterone and estradiol concentrations cannot be measured interchangeably by CMIA and ECLIA.

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T-13

Verification of Diazyme Laboratories human free light chain assays on Alinity ci

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Introduction: Measurements of free light chains (FLCs) are currently used in routine diagnostics of multiple myeloma and the usefulness of FLCs as biomarkers of monoclonal gammopathies is well-known. Furthermore, recent studies are considering FLCs as new potential biomarkers of other disorders in which an inflammatory background has been observed. The aim of the study was analytical verification of Diazyme Laboratories latex particle enhanced immunoturbidimetric assay for the quantitative determination of FLCs in serum (Diazyme Laboratories, Gregg Court Poway, USA) on the Alinity ci analyzer (Abbott Diagnostics, Chicago, USA).

Materials and methods: Analytical verification included precision testing, such as repeatability, day-to-day precision and total precision, performed on quality control materials following CLSI guidelines EP15-A3. Accuracy was estimated from day-to-day precision data. Reference interval (RI) verification was performed according to the CLSI EP28-A3c guidelines using a small number of healthy reference individuals (N = 20).

Results: Obtained repeatability coefficients of variations (CVs) were 2.8 and 1.5% for κ FLC and 1.9 and 1.3% for λ FLC, day-to-day precision CVs were 1.7 and 0.8% for κ FLC and 0.8 and 0.3% for λ FLC and total precision CVs were 3.0 and 1.6% for κ FLC and 1.9 and 1.2% for λ FLC. Both analytes met the acceptance criteria of biological variation for accuracy and precision testing. RIs verification fulfilled acceptance criteria of 18/20 (19/20 healthy reference individuals in manufacturer's RIs both for κ FLC and λ FLC).

Conclusion: The results obtained confirm that the evaluated Diazyme Laboratories latex particle enhanced immunoturbidimetric assays for the quantitative determination of FLCs in serum on the Alinity ci analyzer satisfy the analytical acceptance criteria and can be used in routine practice. Manufacturer's RIs for FLCs can be transferred to our population and analytical platform.

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T-14

Interference of hemolysis on serum protein electrophoresis on MiniCAP Flex Piercing

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Introduction: Hemolysis is a major source of analytical interference in clinical laboratories and is frequently underestimated by visual assessment, while supporting validation data from manufacturers remain limited or insufficiently documented. In serum protein electrophoresis, hemolyzed samples may generate artifacts, including double alpha-2 fraction, and are therefore avoided. We aimed to evaluate the effect of hemolysis on serum protein electrophoresis.

Materials and methods: Hemolysate was prepared from whole blood sample according to CLSI EP7-A2. The sample was first centrifugated at 1500xg for 10 minutes, afterwards the supernatant was carefully removed. The remaining packed cells were washed with an equal volume of 0.9% sodium chloride solution. Washing step was repeated four times to ensure adequate removal of plasma components. Subsequently, 1 mL of distilled water was added to induce erythrocyte lysis *via* osmotic shock. Serum aliquots were spiked with different volumes of hemolysate to obtain final hemoglobin (Hgb) concentration of approximately 0.7, 1, 1.25, 1.75, and 5 g/L. Afterwards, concentration of free Hgb in each sample was measured using the UV-1900i spectrophotometer (Shimadzu, Kyoto, Japan). Serum protein capillary electrophoresis was performed by MiniCAP Flex analyzer (Sebia, Lisses, France). Mean values from duplicate measurements of each protein fraction (%) were used to calculate the biases from native samples. Total error (EFLM biological database) for total proteins and albumin (5%) was used as acceptance criteria.

Results: Bias of alpha-2 fraction exceeded acceptance criteria at concentration of free Hgb at 0.7 g/L. Beta fraction decreased gradually and bias exceeded criteria at 1.25 g/L of free Hgb. Albumin and gamma fraction exceeded acceptance criteria in very hemolyzed samples with 5 g/L of free Hgb. Alpha-1 fraction was unaffected by hemolysis.

Conclusion: Slightly hemolyzed samples should be avoided for serum protein electrophoresis, as alpha-2 fraction increases above acceptable bias in samples with free Hgb of 0.7 g/L.

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T-15

Verification of Abbott Alinity c high-sensitive C-reactive protein assayLucija Drinovac, Tamara Bašić, Erika Jurčić-Karlović, Helena Čičak, Andrea Saračević

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Introduction: High-sensitive C-reactive protein (hs-CRP) is an assay that measures low levels of CRP (0.4 - 160 mg/L) to detect chronic, low-grade inflammation, primarily used to assess cardiovascular disease risk. This study's aim was to verify the Abbott Alinity c hs-CRP assay before its implementation in routine clinical practice.

Materials and methods: Verification protocol included imprecision and accuracy. Measurements were conducted on two Abbott Alinity c analyzers at University Hospital Sveti Duh using control materials and a pool of leftover serum samples. Imprecision was tested using level 1 CRP Vario HS Control Kit and level 2 and 3 Technopath Multichem S Plus controls. The coefficients of variations (CV) were compared with those in the Alinity c CRP Vario Reagent Kit package insert and with the optimal analytical CV from the EFLM Biological Variation Database (EFLM-BV). Accuracy was tested in two different ways. Firstly, according to CLSI EP15-Ed3-WB guidelines and secondly by comparing two analyzers with 40 serum samples using Pasing Bablok and Bland-Altman analysis.

Results: Both analyzers' within-run and within-laboratory CV for level 1, 2 and 3 of control materials were acceptable when compared to the data in the package insert (12.5%, 1.6% and 0.9% respectively), including the optimal CV according to the EFLM-BV (8.7%). The observed bias for Alinity 1 was 0.023 mg/L for level 1, 0.090 for level 2 and 0.431 for level 3 (all < 11.7%). The observed bias for Alinity 2 was 0.018 mg/L for level 1, 0.054 for level 2 and 0.731 for level 3 (all < 11.7%). Passing Bablok analysis showed a statistically significant proportional bias (0.9%) between the Alinity 1 and 2 that is not clinically significant.

Conclusion: Since Abbott Alinity c hs-CRP assay showed acceptable performance characteristics, it has successfully been implemented into daily routine practice.

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T-16

Calcium vs Calcium2 reagent assay: analytical performance comparison on Abbott Alinity c

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Introduction: Although most calcium is stored in the skeletal system, serum calcium plays key physiological role, including reduction of neuromuscular excitability, participation in the coagulation process and activation of certain enzymes. Accurate and precise measurement of serum calcium is therefore essential. The Calcium and Calcium2 assays are both used for the quantitative determination of calcium in human serum on the Alinity c analyzer (Abbott Laboratories, USA). This study aims to evaluate which reagent assay demonstrates better analytical performance.

Materials and methods: Evaluation of both reagent assays included precision, accuracy and reference interval (RI) assessment. Precision was evaluated according to CLSI EP05-A3 guidelines by using two Technopath Multichem S Plus control levels and pooled serum, with coefficients of variation (CV) calculated. The acceptance criteria was 1.4% (EFLM Biological Variation Database). Accuracy was evaluated using 40 patient serum samples analyzed with the Calcium assay and subsequently with the Calcium2 assay. Passing Bablok and Bland Altman analyses were used for method comparison. RI verification was performed using 20 residual serum samples. It was accepted if at least 18 samples fell within the RI range.

Results: The CVs for the Calcium assay were 1.1%, 1.0% and 1.5% for two control levels and pooled serum, respectively. For the Calcium2 assay, the corresponding CVs were 1.7%, 1.6%, and 2.1%. Passing Bablok regression analysis showed no statistically significant difference between assays. Bland Altman analysis demonstrated a proportional bias of 1.1%. This deviation was within the predefined acceptance criteria and was not considered clinically significant. RIs were verified for both reagent assays.

Conclusion: All evaluated performance characteristics were successfully verified, except for precision of Calcium2 reagent assay, which did not meet the EFLM acceptable criteria. Therefore, Calcium reagent assay is more suitable for routine calcium measurement.

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T-17

Method comparison of two osmometers and calculated osmolality in serum and urine samplesZrinka Drenšek Brletić^{1,2}, Željka Vogrinc^{3,4}, Vlasta Cigula Kurajica³, Miljenka Maradin¹¹Department of Medical Biochemistry Laboratory, General Hospital Karlovac, Karlovac, Croatia²Faculty of Kinesiology, University of Zagreb, Zagreb, Croatia³Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia⁴Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Introduction: Measurement of osmolality and calculation of the osmolal gap are useful diagnostic tools in the differential diagnosis of disorders related to water and electrolyte balance, renal function, and small-molecule poisonings. They provide important information on osmotic homeostasis. This study aimed to compare two osmometers in serum and urine samples, and to compare serum results from both osmometers with calculated osmolality method.

Materials and methods: The comparison included 40 serum and urine samples analysed using OsmoPRO (Advanced Instruments, Norwood, Massachusetts, USA) at University Hospital Zagreb and Osmomat 3000 (Gonotec GmbH, Berlin, Germany) at General Hospital Karlovac, as well as calculated osmolality on the Alinity CC system (Abbott, Abbott Park, USA). The results were evaluated using MedCalc statistical software ver.10.0.2.0 (MedCalc Software Ltd, Ostend, Belgium), applying Passing-Bablok regression and Bland-Altman analysis.

Results: Passing-Bablok regression analysis demonstrated excellent agreement between the two osmometers for both urine ($y = 1.00x - 1.93$) and serum samples ($y = 1.00x - 3.00$). In contrast, comparison between calculated osmolality and direct osmometer measurement demonstrated proportional and constant bias for both OsmoPRO ($y = 0.89x + 19.07$) and Osmomat 3000 device ($y = 0.87x + 28.56$). Bland-Altman analysis showed good agreement for the two osmometers in urine (0.2%; 95% CI: -0.15 to 0.56) and serum samples (1.01%; 95% CI: 0.79 to 1.23). Greater deviation was observed between calculated and directly measured osmolality for OsmoPRO (3.67%; 95% CI: 3.31 to 4.02) and Osmomat 3000 (2.68%; 95% CI: 2.31 to 3.05).

Conclusion: The two osmometers showed good agreement in both serum and urine samples. The calculated osmolality method in serum showed slightly lower values compared to direct measurements. However, the differences were small and within clinically acceptable limits, suggesting good overall agreement for routine use and interpretation of the osmolal gap.

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T-18

Comparison study of aldosterone and renin on two automated immunochemical platforms: IDS-iSYS and Maglumi X6Dragana Šegulja^{1,2}, Lucija Balić², Saša Kralić-Ogrić¹¹Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia²University of Applied Health Sciences, Zagreb, Croatia

Introduction: Renin and aldosterone are two of the key regulators for arterial blood pressure, cardiovascular function, and kidney function. The study aimed to compare measurements of renin and aldosterone by two chemiluminescent immunoassays (CLIA) - on the routinely used IDS-iSYS (Immunodiagnostic Systems, United Kingdom) and Maglumi X6 (Snibe Diagnostics, China) analyzers.

Materials and methods: Analyses were performed on 30 routine samples with requests for aldosterone and renin measurements. Parallel measurements on both platforms were run on the same day. Comparison results are presented using Passing Bablok and Bland-Altman statistics (MedCalc Software Ltd., Ostend, Belgium).

Results: The median of renin concentrations was 19 (min. 10 - max. 238) mIU/L. Minor systematic and proportional differences are found (slope 0.85 (95% CI 0.76 - 0.99); intercept 3.37 (0.64 - 5.78); $R = 0.94$ with $P < 0.05$). The mean of differences was 4.45 (- 24.17 to 33.0) mIU/L. Aldosterone concentrations ranged from 41 to 1683 pmol/L, with a median of 384 pmol/L. Passing Bablok statistics revealed significant systematic differences (slope 0.81 (95% CI 0.60 - 0.97), intercept - 77.8 (- 126.9 to -19.17), and $R = 0.90$ with $P < 0.05$). Aldosterone values were higher on the IDS-iSYS analyzer with a mean difference of 153 (- 63 to 370) pmol/L.

Conclusion: On the compared immunochemical platforms, renin results were found to be more comparable than aldosterone results. The compared renin methods, traceable to WHO standard 68/356, show expected differences between different immunoassays, while discrepancies in the compared aldosterone values confirm the absence of an international standard protocol. Our results highlight the significance of standardization in laboratory medicine once again. Following the results of this study, the introduction of a new method for measuring aldosterone in routine practice should include good laboratory practice: informing the clinicians, ensuring parallel measurements, and determining the reference interval or at the least assessing its suitability for the local population.

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T-19

Comparison of urine albumin-to-creatinine ratio determined on Siemens Atellica 1500 and Roche Cobas c503 analyzers

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Introduction: The urine albumin-to-creatinine ratio (ACR) in spot urine samples is recommended by KDIGO guidelines for the clinical grading of albuminuria severity categories (A1-A3). Timely prescribing of combined dual therapy with antihypertensives (ACEIs/ARBs) and antihyperglycemics (SGLT2 inhibitors) can preventively delay the progression of kidney failure by modulating glomerular hemodynamics, thereby enhancing renal protection in patients with confirmed albuminuria. This study aimed to compare the analytical and diagnostic performance of ACR screening between the Siemens Atellica 1500 (Siemens Healthineers, Tarrytown, USA) and Roche Cobas c503 (Roche Diagnostics, Basel, Switzerland) analyzers.

Materials and methods: A total of 122 patients (aged 1 to 85 years) were enrolled in this prospective cohort study in May 2026. Urinary creatinine and albumin concentrations were analyzed on the Roche Cobas c503 using the gold-standard quantitative immunoturbidimetric method for albumin and the Jaffe method for creatinine. Simultaneously, dry-chemistry semiquantitative analyses were performed on the Siemens Atellica 1500 using an albumin-sensitive dye-binding method for albumin and a modified Benedict-Behre method for creatinine, both based on dry-chemistry reflectance photometry. Statistical analysis was performed using MedCalc software (version 14.8.1).

Results: The diagnostic efficiency of the dry-chemistry method for ACR as an albuminuria screening parameter was acceptable for restricted clinical use such as general screening, assessed using receiver operating characteristic curve (ROC) analysis (AUC = 0.85; 95% CI = 0.77 - 0.91; Sn = 98.4% and NPV = 96.8%). Intercategorical agreement for albuminuria among the A1-A3 subgroups, assessed using quadratic weighted Kappa statistics, revealed a substantial degree of agreement (quadratic weighted kappa = 0.64; 95% CI = 0.55 - 0.73).

Conclusion: Widespread clinical implementation of the dry-chemistry ACR screening method has an important public health impact and may facilitate rapid screening classification of albuminuria stages, potentially preventing the progression of albuminuria-induced nephrotoxicity through the early detection of renal injury. Consequently, laboratory albuminuria screening is pivotal for reducing cardio-cerebrovascular risk and delaying the progression of cardiorenal impairment.

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T-20

Comparison of Sampson-NIH and Friedewald equations for LDL-C estimation against direct LDL-C measurement across different triglyceride concentrations

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Introduction: Calculated low-density lipoprotein cholesterol (LDL-C) is widely used when direct measurement is unavailable. This study compared the Friedewald formula (FF) and the recently recommended Sampson-NIH equation (SF) with directly measured LDL-C (dLDL-C) across different triglyceride (TG) concentrations.

Materials and methods: This retrospective study included serum samples submitted for routine lipid panel analysis between January 2024 and December 2025. Data were obtained from the laboratory information system (LIS). Concentrations of cholesterol, TG, and dLDL-C were measured on the Abbott Alinity c platform, with dLDL-C used as the reference method for comparison. LDL-C was calculated using the Friedewald formula and the Sampson-NIH equation (2020) adapted for SI units (mmol/L). Agreement between calculated and dLDL-C was evaluated using Bland–Altman (BA) analysis and Passing–Bablok (PB) regression in MedCalc v20.013 (Ostend, Belgium).

Results: A total of 16,725 samples were stratified into three TG groups: < 1.7 mmol/L (N = 7001), 1.7 - 4.1 mmol/L (N = 9205), and > 4.1 mmol/L (N = 519). Percentage BA bias for FF progressively increased with rising TG concentrations from 3.43% to 5.65% and 20.86%, whereas SF showed consistently lower bias across all TG groups (2.48%, 3.59%, and 11.29%, respectively). PB regression demonstrated smaller systematic and proportional differences for SF across all TG groups. In hypertriglyceridemic samples (TG > 4.1 mmol/L), PB equations were $y = -1.12 (-1.27 \text{ to } -0.98) + 1.15 (1.10 \text{ to } 1.19) x$ for FF and $y = -0.14 (-0.25 \text{ to } 0.00) + 0.94 (0.91 \text{ to } 0.98) x$ for SF.

Conclusion: The Sampson equation demonstrated better agreement with dLDL-C than the Friedewald formula, particularly at elevated triglyceride concentrations, supporting its use as a more reliable alternative for routine LDL-C estimation.

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T-21

Analytical verification and method comparison of alpha-1-antitrypsin measurement on an immunonephelometric analyzer

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Introduction: Alpha-1-antitrypsin (A1AT) measurement is routinely used in the diagnostic evaluation of A1AT deficiency and inflammatory conditions. The aim of this study was to verify the analytical performance of A1AT measurement on an immunonephelometric analyzer and compare it with an immunochemistry-based method.

Materials and methods: Analytical verification was performed on the AtellicaNEPH630+ immunonephelometric analyzer (Siemens Healthineers, Germany). Method comparison was performed against the Alinity-i immunochemistry analyzer (Abbott Laboratories, Abbott Park, USA). Imprecision was assessed using N/T PROT CONTROL SL/L and SL/M, measured in five replicates over five consecutive days. Within-laboratory precision, bias and allowable total error (TE) were calculated. Acceptance criteria were based on desirable biological variation specifications for A1AT according to Ricos *et al.* and the Westgard database: imprecision $\leq 3.0\%$, bias $\leq 4.3\%$ and allowable total error $\leq 9.2\%$. Method comparison included 15 serum samples and was evaluated using Passing-Bablok regression and Bland-Altman analysis. Clinical relevance of inter-method difference was assessed using reference change value (RCV), calculated from analytical variation and within-subject biological variation, with CVI of 3.8% obtained from the EFLM Biological Variation Database. Statistical analysis was performed using MedCalc software (version 14.8.1, Ostend, Belgium).

Results: Mean A1AT concentrations of the two control levels were 0.91 g/L and 1.62 g/L. Within-laboratory coefficients of variation were 2.35% and 3.47%, with a mean value of 2.91%. Bias was - 2.23% and - 1.89%, with a mean bias of - 2.06%. Allowable TE was 4.71% and 6.94%, with a mean value of 5.83%. Passing-Bablok regression yielded $y = - 0.15 (- 0.27 - 0.04) + 1.09 (0.96 - 1.17) x$. Bland-Altman analysis showed a mean relative difference of - 1.9%, below the calculated RCV for decrease (- 12.4%).

Conclusion: A1AT measurement on the AtellicaNEPH630+ demonstrated acceptable analytical performance. Method comparison showed good agreement with Alinity i, without statistically significant constant or proportional error. The observed inter-method difference was below the calculated RCV, indicating also no clinically significant difference between methods.

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U Other

U-01

Vitamin D status and supplementation in patients with spinal cord injury

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Introduction: Patients with spinal cord injury (SCI) are at increased risk for vitamin D insufficiency/deficiency because they don't have enough exposure to sunlight due to the immobility, low physical activity and bed-rest. An insufficiency (vitamin D concentration 50-75 nmol/L) and deficiency (vitamin D concentration < 50 nmol/L) should be treated with vitamin D supplementation. However, there is a lack of evidence regarding the optimal dosage and duration of vitamin D supplementation in patients with SCI. The aim was to determine vitamin D status in patients with SCI and based on it determine whether it is necessary to make changes in vitamin D supplementation.

Materials and methods: The study included serum samples from patients with acute (N = 35) and chronic SCI (N = 14). 25-OH vitamin D (25(OH)D) was measured on Snibe Maglumi X3 using CLIA method. Supplementation data were taken from the hospital information system by the clinical pharmacist. Statistical analysis was performed with MedCalc (MedCalc Software, Ostend, Belgium).

Results: In patients with acute SCI mean concentration of 25(OH)D was 57.4 ± 35.1 nmol/L. Insufficiency/deficiency was present in 68% patients of which 25% use vitamin D supplementation. In patients with chronic SCI mean concentration of 25(OH)D was 62.5 ± 44.8 nmol/L. Insufficiency/deficiency was present in 64% patients of which 55% use vitamin D supplementation.

Conclusion: In patients with acute and chronic SCI there is high prevalence of vitamin D insufficiency/deficiency. Since vitamin D insufficiency/deficiency is related to number of health issues, for example osteoporosis, which is widely spread among patients with SCI, there is need for implementation of vitamin D supplementation in all patients with SCI, periodic determination of vitamin D status and correction of dosage if necessary.

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U-02

Evaluation of diagnostic accuracy of semi-quantitative urine test strips for routine screening of albumin/creatinine ratio in urine

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Introduction: For initial testing of albuminuria, current guidelines recommend quantitative measurement of the urinary albumin-to-creatinine ratio (ACR) or reagent strip urinalysis for albumin and ACR with automated reading. The aim of this study was to evaluate the diagnostic accuracy of semi-quantitative urine test strips for ACR compared to a quantitative reference method.

Materials and methods: In this study, 133 urine samples were randomly selected and analyzed. Semi-quantitative measurements were performed using the CLINITEK Novus Analyzer with Pro 12 urine dipsticks (Siemens Healthineers, Erlangen, Germany), while the reference quantitative method was performed on the Alinity c analyzer (Abbott, Abbott Park, USA). Both analyses were performed on the same samples within a two-hour interval. The cut-off values for classification were defined as 2 mg/mmol for the dipstick method (according to the manufacturer's instructions) and 3 mg/mmol for the quantitative method (according to current guidelines). Diagnostic performance parameters, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated. Agreement between methods was assessed using the weighted kappa coefficient. Statistical analysis was performed using MedCalc software, v22.006 (Ostend, Belgium).

Results: Albuminuria was detected by both methods in 52 patients. A total of 28 false-positive and no false-negative results were observed in semi-quantitative measurements. The results show moderate agreement (weighted kappa = 0.60; 95% CI 0.48 - 0.72). The following values were calculated: sensitivity 100%, specificity 65%, PPV 65%, NPV 100%, and accuracy 79%.

Conclusion: Given the high sensitivity and high NPV, semi-quantitative test strips demonstrate adequate diagnostic characteristics for albuminuria screening and can serve as an effective rule-out tool, with quantitative confirmation of all positive/borderline findings. This two-step approach ensures faster results and rational use of laboratory resources.

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U-03

Inverse association between HbA1c and vitamin D in female patients but not in males: a retrospective study

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Introduction: A growing body of observational evidence suggests an inverse relationship between glycated hemoglobin (HbA1c) and serum vitamin D concentrations in patients with type 2 diabetes and related metabolic conditions, although findings remain heterogeneous and influenced by population-specific factors. The potential role of gender as an effect modifier in this relationship has not been consistently explored. Therefore, the aim of this study is to evaluate the association between HbA1c and vitamin D concentrations and to determine whether this relationship differs between male and female patients.

Materials and methods: A retrospective cross-sectional analysis was conducted on patients with available HbA1c and serum vitamin D measurements. Correlation between HbA1c and vitamin D was assessed using Pearson and Spearman coefficients. Analyses were performed for the overall cohort and stratified by gender. Statistical significance was defined as $P < 0.05$.

Results: No statistically significant correlation between HbA1c and vitamin D concentrations was observed in the overall cohort. However, gender-stratified analysis revealed a differential pattern. Among male patients, the correlation was weak and not statistically significant (Pearson $r = -0.09$, $P = 0.18$). In contrast, female patients demonstrated a statistically significant inverse correlation (Pearson $r = -0.21$, $P = 0.01$; Spearman $r = -0.24$, $P = 0.006$), indicating that lower vitamin D concentrations were associated with higher HbA1c values in this subgroup.

Conclusion: While no overall association between HbA1c and vitamin D was identified, a significant inverse relationship was observed in female patients but not in males. These findings are consistent with prior observational data suggesting an inverse HbA1c - vitamin D relationship, while highlighting gender as a potential effect modifier. Further studies incorporating additional confounders such as age, body mass index, and comorbidities are warranted to clarify underlying mechanisms and clinical implications.

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U-04 (Oral presentation)

Toward establishing updated national reference intervals in Croatia: a pilot study by the Croatian Chamber of Medical Biochemistry using alkaline phosphatase as a model analyte

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Introduction: Despite demographic changes and continuous analytical advancements, Croatian national reference intervals (RIs) have not been updated since 2007. To address this gap, the Croatian Chamber of Medical Biochemists initiated a nationwide pilot study to evaluate the feasibility of indirectly derived RIs. Our aim is to present calculated RIs for alkaline phosphatase (ALP) as a model analyte to demonstrate the approach and to compare them with those currently in use.

Materials and methods: Seven laboratories participated in this pilot study. Following ethics committee approval, data for 2021–2025 were extracted from three different laboratory information systems and harmonized to address structural and formatting differences. Approximately 25 million results for 25 analytes were collected. Among these, ALP results measured on Roche cobas analyzers (Mannheim, Germany) were available from 5 of the 7 participating laboratories. Indirect ALP RIs were estimated using the RefineR package (v2.0.0) and compared with current national RIs (60 - 142 IU/L for males $\geq$ 20 years, 54 - 119 IU/L for females aged 20 - 50 years, and 64 - 153 IU/L for females $\geq$ 50 years).

Results: Of 973,652 ALP results, 99,255 were retained after technical data cleaning and filtering, representing individuals from birth to 100 years of age. The estimated RIs were 44 - 117 IU/L for males $\geq$ 20 years ($N = 35,543$), 35 - 97 IU/L for females aged 20 - 50 years ($N = 22,081$), and 46 - 125 IU/L for females $\geq$ 50 years ($N = 29,007$). For each subgroup, the estimated non-pathological fraction exceeded 95%. Stratification by sex and age was consistent with the current national RIs.

Conclusion: Indirectly derived RIs for ALP are lower than the currently applied national intervals, suggesting that current limits may be overestimated. This pilot study demonstrates that indirect methods are feasible for updating national RIs in Croatia. Further analyses, including additional laboratories and analytical platforms, are required before implementation.

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U-05

Evaluation of processed specimen stability for T-, B- and NK-lymphocyte subset analysis by flow cytometry

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Introduction: Enumeration of T-, B- and NK-lymphocyte subsets by flow cytometry is among the most commonly performed assays in our Cellular Immunology Laboratory. The assay is used for diagnosis and monitoring of primary and secondary immunodeficiencies, immunosuppressive and immunomodulatory therapies, organ transplantation, and viral infections. Current recommendations suggest acquisition of flow cytometry data as soon as possible after sample preparation; however, increasing workload can postpone immediate acquisition. The aim of this study was to evaluate stability of processed (stained, lysed and fixed) samples and determine whether delayed acquisition affects the results.

Materials and methods: Five peripheral blood samples were processed in octuplicate using surface staining protocol with the following fluorochrome-conjugated antibodies: CD4-FITC, CD16/CD56-PE, CD45-PerCP-Cy5.5, CD19-PE-Cy7, CD3-APC and CD8-APC-H7. Samples were acquired on FACSLytic flow cytometer (BD Biosciences, USA) within 1 hour after staining (baseline), and additionally at 2, 4, 6, 8, 16, 22 and 24 hours post-staining. Until acquisition, processed samples were stored protected from light at 4 °C. Data were analyzed using BD FACSuite software version 1.4 to determine relative and absolute lymphocyte subset counts. Sample stability was assessed as the relative deviation from baseline values, using an acceptance criterion of $\pm 20\%$, in accordance with ICSH and ICCS guidelines.

Results: For all lymphocyte subsets, individual and mean deviations at all time points remained below the predefined acceptance criterion of 20%. At 24 hours after staining, mean relative deviations from baseline for CD3(%), CD3+CD4+(%), CD3+CD8+(%), CD19+(%), NK (%), CD3(cells/ μ L), CD3+CD4+(cells/ μ L), CD3+CD8+(cells/ μ L), CD19+(cells/ μ L), and NK(cells/ μ L) were 1%, 4%, 3%, 4%, 10%, 1%, 4%, 3%, 4%, and 10%, respectively.

Conclusion: Both mean and individual deviations remained within the predefined acceptance criterion. Processed samples for T-, B- and NK-lymphocyte subset analysis, stored protected from light at 4 °C, remain stable for up to 24 hours after processing and can be reliably acquired within this time frame.

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U-06

Evaluation of the efficiency of different solvents for the extraction of the amino acids phenylalanine, tyrosine, and methionine from dried plasma spot samples on filter paperIva Bilandžija Kuš¹, Ana Škaričić¹, Ksenija Fumić^{1,2}¹Department for Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia²Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Introduction: Dried plasma spot (DPS) sampling on filter paper, using the Telimmune Plasma Separation Card (North Webster, USA), represents a sample collection technique that, compared with dried blood spot (DBS) sampling, eliminates the influence of hematocrit while maintaining plasma volume accuracy within 5%. However, due to the limited plasma volume recovered in DPS samples, optimization of this sample type for amino acid analysis remains challenging. The aim of this study was to evaluate the extraction efficiency using four different solvents for amino acids phenylalanine, tyrosine, and methionine from DPS samples, and to identify the most suitable extraction solvent.

Materials and methods: Four extraction solvents were investigated: 0.1% formic acid in water, 20% methanol in water, 0.1% formic acid in methanol, and 0.1% formic acid in water/methanol (80/20). Pool samples (N = 30) were prepared by combining residual whole blood specimens from previously analyzed samples. For the evaluation of each extraction solvent, 40 µL of whole blood from each pool was applied to DPS cards, and corresponding plasma samples were prepared. The extraction procedure involved incubation of DPS samples in 50 µL of the respective solvent for 30 minutes on a shaker, followed by 5 minutes in an ultrasonic bath. Extracted DPS samples and plasma samples were processed according to the standard analytical protocol for amino acids analysis.

Results: Data normality was assessed using the Shapiro-Wilk test (normality was rejected). *Post hoc* analysis following the Friedman test demonstrated no statistically significant difference between the concentrations of phenylalanine, tyrosine, and methionine measured in DPS samples extracted with 0.1% formic acid in water and those measured in plasma samples. In contrast, all other extraction solvents showed statistically significant differences ($P < 0.05$).

Conclusion: 0.1% formic acid in water provides the most efficient extraction of phenylalanine, tyrosine, and methionine from DPS samples.

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