What´s floating on my plasma?

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Abstract

We report on a preanalytical issue we encountered during routine clinical chemistry analyses, potentially leading to deviated analysis results and believe that it might help other laboratories to overcome similar problems. In a heparin-gel tube we measured an implausible glucose value of 0.06 mmol/L. Re-measurement of the same sample resulted in a glucose value of 5.4 mmol/L. After excluding an analytical error, we inspected the sample closer and found a white material as well as fatty droplets floating on the surface of the plasma tube. Evaluation of these structures revealed that the white particulate matter (WPM) consisted of fibrinogen, platelets and leukocytes and the fatty droplets most probably originated from the separator gel. We concluded that these structures formed a temporary clot in the instruments pipetting needle thereby altering the sampling volume and subsequently the measured glucose value. The formation of WPM might be attributable to high speed centrifugation, high cholesterol levels, the gel formulation or a combination of several issues such as temperature, heparin concentration, pH and patient-specific factors. The gel droplets were most probably caused by an aberrant gel formulation in combination with an improper storage of the empty tubes on the wards prior to phlebotomy. After adding an additional instrument cleansing cycle and changing to another batch of heparin tubes the problems could be significantly reduced.

Key words: case report; white particulate matter; lithium-heparin tubes; separator gel

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Introduction

With this case report we want to highlight a preanalytical issue, potentially leading to instrument pipette clogging and/or deviated analysis results due to incorrect sample volume aspiration. We believe that it is more frequent than commonly anticipated and that it is of great importance, especially for high throughput laboratories using primary samples on their analysers. For laboratories dealing with similar issues, this report might be helpful to overcome similar problems more quickly.

Laboratory analyses

Our laboratory encountered the following analytical problem using the COBAS Modular P system (Roche Diagnostics, Rotkreuz, Switzerland). A heparin-plasma sample with a separator gel was collected from a patient and centrifuged at 2000 g for 10 minutes after being sent to our laboratory by a pneumatic tube system. Total transportation time of this sample was only 8 minutes. Subsequently the values presented in Table 1 were measured.

Further investigation

As a glucose value of 0.06 mmol/L (1 mg/dL) was clinically implausible, the value was re-measured from the same sample, yielding a glucose level of 5.4 mmol/L (97 mg/dL). Initially, we investigated potential analytical errors for this discrepancy, but as the internal and external quality assurance values were all on target in high and low levels and

glucose values of other patient samples were all inconspicuous, we started to investigate potential pre-analytical errors. While investigating the tube more closely, we noticed clotted white material as well as fat droplets floating on the surface of the sample (Figure 1). Similar observations were made in a small number of additional heparin-gel-tubes of other patients. In some of these tubes we found either fat droplets or white clots (Figure 2) and in some we found a combination of both. Some of the white clots were visible by the naked eye whereas others could only be detected with a digital microscope camera including a light source.

Solution

After staining the white clots with a May-Grünwald-Giemsa stain and evaluating them under the microscope, we identified it as a conglomerate of fibrinogen, platelets (PLT) and some leukocytes (WBC), described as "white particulate matter" (WPM). The fat droplets most probably originated from the separator gel in the tubes. We concluded that these structures floating on the surface of the heparin tubes were sufficient to form a temporary clot in the pipetting needle, whereby the sampling volume was significantly altered. As a result of this deviation, glucose measurement, which only needs 2 µL of plasma, was affected accordingly. After adding an additional instrument routine cleansing cycle throughout the day and an exchange to another heparin tube batch we were able to reduce these issues to a minimum.

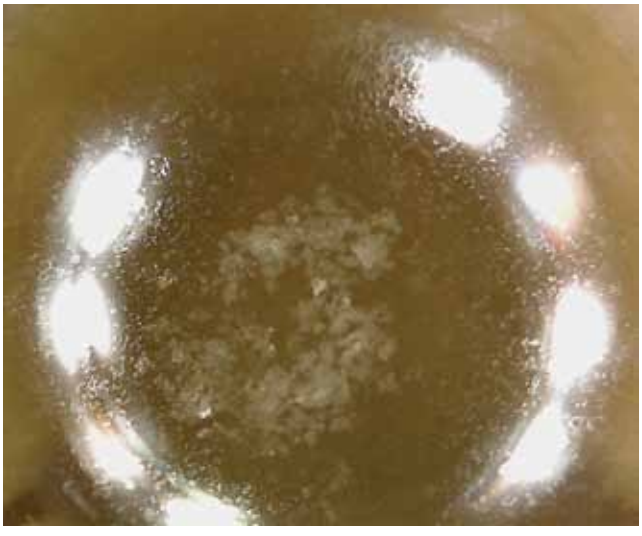

Figure 1. White particulate matter as well as gel droplets floating on the surface of a heparin-plasma tube (picture taken with a USB microscope camera).

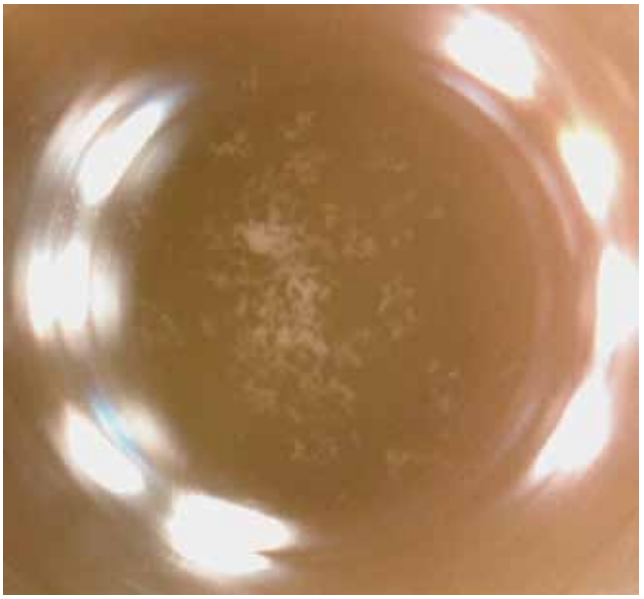

Figure 2. White particulate matter floating on the surface of a heparin-plasma tube (picture taken with a USB microscope camera).

Discussion

We report a case where gel droplets as well as white particulate matter on the surface of heparin gel tubes altered routine clinical chemistry analyses. The formation of so called white particulate matter is a phenomenon which was primarily described in red blood cell units (1-5). In 2003, WPM was detected in blood preparations collected into Terumo blood-collection bags. Bright field and differential interference contrast microscopy revealed that they consisted of cellular debris, WBCs, PLTs, and a few fibrin strands surrounded by erythrocytes. As the United States Food and Drug administration did not conclusively rule out a potential association between WPM formation in blood units and some adverse events, blood units had to be monitored and be quarantined in case of WPM

Table 1. Measurements of case sample

Parameter	Unit	Value Measurement #1	Value Measurement #2	Reference value
Chloride	mmol/L	103	/	97 - 108
Potassium	mmol/L	4.0	/	3.6 - 5.0
Sodium	mmol/L	144	/	135 - 148
Urea	mmol/L	4.0	/	2.8 - 8.1
Creatinine	µmol/L	70.8	/	62 - 106
Calcium	mmol/L	2.28	/	2.13 - 2.63
Glucose	mmol/L	0.06	5.4	3.9 - 5.6
CRP	mg/L	< 6	/	< 6

CRP – C reactive protein.

appearance (6). In one letter from Dimeski *et al.,* appearance of WPM is mentioned in routine samples for clinical chemistry analyses (7). They report intermittent duplicate errors in lactate dehydrogenase measurements using lithium-heparin gel tubes on a Hitachi device due to microclots consisting of leukocytes, erythrocytes, platelets and fibrinogen, causing sampling errors, similar to the ones we report here. The authors refer to 4 to 8% of all heparin-gel tubes containing WPM, depending on the tube manufacturer. They attribute the analytical interference of WPM to several conditions:

1) WPM may cause sampling problems especially in low volume tests as they are detected as part of the total volume. This fits to our finding of an altered glucose value for which only 2 µl of plasma is used.
2) If cellular components of the WPM rupture, they might release intracellular components altering the sample composition.
3) If intact WPM is transferred into the reaction cuvette, it might interfere with the optical system.

The authors consider the gel formulation of the respective tube to be responsible for the formation of these microclots. Patel *et al*., however, suggested that high speed centrifugation and high cholesterol levels might cause WPM (8). Since WPM is primarily detectable in heparin tubes, this anticoagulant most probably is one of the causing agents. Also other parts of the pre-analytical process such as transport or centrifugation conditions or the time from blood collection until centrifugation could be contributing factors. We believe however, that a combination of more than one influencing factor is the reason for WPM formation. Such factors might include temperature, pH, the filling grade of the tube and the subsequently altered final heparin concentration or patient specific factors related to their medical condition. To investigate this topic, a rather elaborate study would be needed to address all these possibilities.

In our case not only WPM was present but also fat droplets on the plasma surface, most probably originating from the separator gel. After casual analysis, we concluded that they formed due to an aberrant gel formulation of the respective tube batch in combination with inadequate storage of the empty tubes at high temperatures on the wards prior to phlebotomy, thereby releasing small amounts of gel components from the gel plug. These droplets then adhered to the inner surface of the pipetting needle and as the gel formations were also found in other clinical samples, an incremental occlusion was the result.

Not all laboratories will experience this problem depending on their equipment and set up. The reasons for this are that either the number of tubes processed between two instrument cleansing cycles is too small to form plaques on the pipetting needle, or they used a pre-analytical system for aliquoting plasma prior to analytics. Thereby WPM

and gel droplets bound to the disposable pipette tip from the pre-analytics module got discarded before being transported to the analytical instruments. This theory was strengthened by the fact that WPM was not of concern in the immunology module of the COBAS instruments (E-Module) which also uses disposable pipette tips for sampling. In contrast to the above mentioned laboratories, all measurements in our clinical chemistry department were performed from primary sample tubes without any pre-analytical handling except centrifugation and decapping, thereby exposing the analytical instrument directly to the interfering substances.

What you should / can do in your laboratory to prevent such errors

For those laboratories who encounter similar problems as the ones we describe, we suggest:

- adding additional routine cleansing procedures to the instrument,
- changing to another heparin tube batch,
- instructing the wards on how to store blood collection tubes properly, or
- adding an aliquotation step prior to transportation to the analytical unit.
- If this doesn't resolve the issue, the use of serum samples instead of heparin plasma samples might be helpful as WPM in these tubes is found at much lower rates (7).

Potential conflict of interest

None declared.

References

1. *Hillyer CD, Roback JD, Hillyer KL, Josephson CD, Page PL. Description and investigation of white particulate matter in additive solution-1 red blood cell units. Transfusion 2004;44:977-83. https://doi.org/10.1111/j.1537-2995.2004.04036.x.*
2. *Iwamoto M, Curns AT, Blake PA, Jernigan DB, Holman RC, Lance-Parker SE, et al. Rapid evaluation of risk of white particulate matter in blood components by a statewide survey of transfusion reactions. Transfusion 2004;44:967-72. https://doi.org/10.1111/j.1537-2995.2004.03319.x.*
3. *McCullough J, Dodd R, Gilcher R, Murphy S, Sayers M. White particulate matter: Report of the ad hoc industry review group. Transfusion 2004;44:1112-8. https://doi.org/10.1111/j.1537-2995.2004.04098.x.*
4. *Rentas FJ, Macdonald VW, Rothwell SW, McFaul SJ, Asher LV, Kennedy AM, et al. White particulate matter found in blood collection bags consist of platelets and leukocytes. Transfusion 2004;44:959-66. https://doi.org/10.1111/j.1537-2995.2004.03398.x.*
5. *Simon TL. White particulate matter: This time too much precaution. Transfusion 2004;44:956-8. https://doi.org/10.1111/j.1537-2995.2004.44702.x.*
6. *Sachdev S, Dhawan HK, Khetan D, Marwaha N, Jain A, Sharma RR. White particulate matter in a packed red blood cells unit. Asian J Transfus Sci 2011;5:175-6. https://doi.org/10.4103/0973-6247.83247.*
7. *Dimeski G, Badrick T, Flatman R, Ormiston B. Roche ifcc methods for lactate dehydrogenase tested for duplicate errors with greiner and becton-dickinson lithium-heparin and greiner serum samples. Clin Chem 2004;50:2391-2. https://doi.org/10.1373/clinchem.2004.039248.*
8. *Patel HD, Byrne KM, Horne M, Leitman SF, Stroncek DF. Factors affecting the formation of white particulate matter in red blood cell components. Transfusion 2005;45:1127-32. https://doi.org/10.1111/j.1537-2995.2005.04351.x.*