

## Original papers

### Evaluating performance in sweat testing in medical biochemistry laboratories in Croatia

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#### Abstract

**Introduction:** Sweat test has a diagnostic role in evaluation of cystic fibrosis. Its performance includes sweat stimulation, collection and analysis. All listed may be sources of inconsistencies in everyday practice. The aim of this study was an evaluation of external quality assessment (EQA) of sweat chloride measurement including sweat test performance in medical biochemistry laboratories in Croatia.

**Materials and methods:** EQA for sweat chloride measurement was provided by Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) in five consecutive exercises to medical biochemistry laboratories (MBL) that offered sweat testing. A questionnaire regarding all phases of testing was mailed to involved MBL (N = 10). Survey results were compared to current guidelines for sweat test performance.

**Results:** Reported results of EQA in 2015 exercises showed coefficients of variation (CV) from 28.9%, 29.0% to 35.3%, respectively. An introduction of uniform sweat chloride measurement protocol resulted in CV of 15.5% and 14.7% reported in following two exercises in 2016. All MBL included in this study replied to the questionnaire. Results reported by MBL indicated: lack of patient information policy (7/10), use of unacceptable electrodes (6/9), misuse of minimum of acceptable sweat weight (6/9), lack of internal quality assessment (5/9) and recommended reference ranges (5/9 and 4/9). Agreements to guidelines were found in approach to unsuitable patients (9/10) and sweat collection (8/9).

**Conclusion:** Presented results indicate major weak points of current practice in sweat test performance in Croatian MBL and stress the need for its standardization on a national level.

**Key words:** sweat testing; external quality assessment; survey

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#### Introduction

It was six decades ago since Gibson and Cook introduced sweat testing in clinical practice for diagnosis of cystic fibrosis (CF) (1). It is an inherited, life-shortening disease mostly presented by dysfunction of gastrointestinal and respiratory organs caused by mutation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene (2). CFTR gene regulates translation of CFTR protein, ubiquitous membrane regulator presented also on epithelium of sweat glands and involved in transport of chloride, sodium and water (3). CF incidence and prevalence vary across Europe, but a median

incidence is around 1:3500 and a mean prevalence of 0.737 per 10,000 indicating that prevalence is less than 5 per 10,000 and classifying CF in rare or "orphan disease" (4,5). Demographic data of CF in Croatia (south-east Europe, population of 4,290 000 roughly, birth rate around 39,000 per annum) are rather poor giving an estimated number of around 110 individuals affected by disease (6,7).

The sweat test is three steps procedure including sweat gland stimulation, sweat collection and chloride measurement. Through decades sweat test evolved in variety of techniques for sweat

stimulation, collection and analysis, but a basic principle of sweat gland stimulation by pilocarpine iontophoresis (PI) remained unchanged. Currently there are two accepted techniques for sweat stimulation and collection in the clinical settings: Gibson and Cook technique (GCT) and Wescor Macroduct collection system (8,9). The latter is a commercial set while GCT includes verified set of devices and materials according to international official regulation for PI (9). Both of them require manual work and extensive skills in the performance. According to current guidelines the acceptable method for sweat chloride measurement can be any of listed: coulometry, indirect ion selective electrode (ISE), mercurimetric titration (Schales and Schales micro method) if is validated (8,9). External quality assessment data indicate domination of coulometry over indirect ISE or titration (10). Recently, an inductively coupled plasma mass spectrometry was reported as a promising method for sweat chloride quantification (11). Guidelines approved methods take part of sweat testing intended for diagnosis of CF. In clinical practice was also adopted the sweat conductometry method that used PI for sweat glands stimulation but measured conductivity (concentration and mobility) of chloride and all other ions in sweat (8). Due to its lower diagnostic accuracy than sweat test, the sweat conductometry was given role of screening test for CF (9).

Croatian national guideline for CF diagnosis and management is in line with the international standards giving a diagnostic role to sweat test in CF clinical pathway (12). In era of published international sweat test performance guidelines it seems that inconsistencies in carrying out a sweat test are still present in everyday practice (13,14). Considering published reports about real-life sweat test challenges, a question about national sweat test practice emerged in light of related data lack. It prompted the Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) to introduce in 2015 the Sweat test scheme as a pilot for screening a current practice of that test in Croatia (15). The CROQALM is a national provider of external quality assessment (EQA) for all registered medical biochemistry laboratories (MBL) in Croatia. Participation in all schemes ex-

cept pilots is mandatory for all MBL according to policy of the Croatian Chamber of Medical Biochemist (16). The Sweat test scheme has been covering only the analytical phase of the test and data collected in 2015 show unacceptable analytical outcomes with large data dispersions. It called for an urgent action and raised questions about other topics in sweat test performance. All together it made a path to survey about real-life sweat test practice in Croatia with the final goal to detect critical points of sweat test procedure.

## Materials and methods

In 2015, the CROQALM database registered 196 MBL, eight of them (8/196; 4%) performed sweat chloride measurement by mercurimetric titration. In 2016 one more MBL participated in sweat test scheme (9/198; 5%). During 2015 and 2016 one MBL offered sweat conductometry which results were not included in EQA evaluation but that MBL took part in the survey. Finally, total number of MBL participated with results in sweat test scheme was 8 in 2015 and 9 in 2016 but total number of survey participants was 10. In the first exercise (June 2015) one control sample (liquid ready to use) for sweat test scheme was delivered by mail (written instruction included) to all MBL registered in CROQALM database. In September 2015 exercise as well as in all following CROQALM rounds, control sample was distributed only to MBL reporting sweat chloride result in previous scheme. Participating laboratories reported data on-line by the link on CROQALM website.

Reported results from two exercises (June and September) in 2015 were far from acceptable. Considering those poor data, we asked sweat test scheme participating MBL to send to CROQALM their written protocols for the chloride measurement in sweat. Comparing received protocols from eight MBL we found many inconsistencies in relation to the original Schales and Schales micro method, the protocol for sweat chloride mercurimetric titration (17). Therefore, sweat test team prepared a uniform protocol in accordance to above listed method and mailed it with a questionnaire in the first 2016 exercise (March 2016) to all MBL participating in sweat test scheme including MBL

that performed sweat conductometry. Beside three previously listed exercises, sweat test scheme participated in two additional CROQALM exercises in November 2015 and June 2016.

The questionnaire was mailed in second exercise 2016 (May 2016) to all MBL offering sweat test. It encompassed MBL participating in sweat test scheme and one MBL offering sweat test conductometry. Total number of involved MBL was ten.

Mailed questionnaire was redesigned and enlarged version of similar set of questions that had been used by the Working group for external body fluids of CSMBLM in 2015 survey. Data regarding sweat test collected in that study had never been published due to poor response rate of the participated Croatian MBL (18).

The questionnaire comprised of 18 questions (Q1-Q18) intending to provide data on key points of sweat test performance (pre-analytical, analytical and post-analytical phases). Questionnaire was designed with multiple choices of responses and a possibility to specify an answer. Additional request a photo of the electrodes that participants use for sweat stimulation was also mailed. Total of 16 questions referred to contents of internationally accepted guidelines. The other two questions (Q3, Q9) had local features due to informal and unpublished data about real-life sweat test practice in Croatia. Participation in the survey was voluntary and the results were coded.

The control sample for CROQALM sweat testing scheme is "in house" chloride solution prepared just prior to each CROQALM exercise in the Clinical Institute of Laboratory Diagnostics, University Hospital Centre Rijeka according to protocol for internal quality control (8). Results of sweat test scheme were evaluated using inlab2\*QALM software (IN2 Group Ltd., Zagreb, Croatia), specifically designed in 2011 for quality evaluation of medical laboratory performances. Outliers were excluded manually for results 40% below or above of the all participants group mean.

Results of the survey are presented in absolute numbers.

## Results

### CROQALM exercises 2015-2016

In 2015 results of chloride measurements by mercurimetric titration had been received from eight MBL but in 2016 one more MBL was introduced in sweat test scheme. In conclusion total of 9 MBL were included in CROQALM sweat test scheme in 2016.

In five CROQALM exercises (2015-2016), mean and median chloride concentrations in control samples ranged from normal (< 30 mmol/L) to intermediate (< 60 mmol/L) levels regarding chloride reference ranges in sweat. In 2015 reported data from MBL extended from normal to positive ranges (> 60 mmol/L) with coefficients of variation

**TABLE 1.** Results of Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) exercises in chloride concentrations of the sweat test scheme

CROQALM exercises	N	Chloride concentrations in control samples			Outliers
		Mean $\pm$ SD (mmol/L)	Median (range) (mmol/L)	CV (%)	
<b>Before uniform protocol introduction</b>					
1 <sup>st</sup> (June 2015)	8	53.0 $\pm$ 15.0	51.5 (36.3 – 128.0)	28.9	0
2 <sup>nd</sup> (September 2015)	8	25.5 $\pm$ 7.4	23.9 (16.0 – 36.0)	29.0	1
3 <sup>rd</sup> (November 2015)	8	45.4 $\pm$ 16.1	52.8 (22.5 – 64.6)	35.3	3
<b>After uniform protocol introduction</b>					
1 <sup>st</sup> (March 2016)	8	29.7 $\pm$ 4.6	28.5 (24.8 – 37.0)	15.5	0
2 <sup>nd</sup> (June 2016)	9	21.5 $\pm$ 3.2	19.9 (18.0 – 26.0)	14.7	2

N – Number of participating laboratories. SD - standard deviation. CV – coefficient of variation.

from 28.9% to 35.3%. An introduction of uniform sweat chloride mercurimetric titration protocol for all MBL involved in Sweat test scheme resulted in reported coefficients of variation from 14.7 to 15.5% in next two exercises in 2016 (Table 1).

## Survey

The response rate on survey was maximal in sense that all involved MBL (N = 10) replied (Table 2), but passed over the request about sending electrodes photos. They were collected by an addition request to all involved MBL during the third CROQALM exercise in 2016. One MBL that performed sweat con-

ductometry responded on 10 out of 18 questions (Q1-Q8, Q13 and Q15). All of them addressed preanalytical phase of sweat testing that is common to two previously listed sweat test varieties. On Q8 one MBL replied with a comment but without an answer. On Q16 and Q13 response rates were 8/9 and 9/10 MBL. On Q14 participants specified as follows: "in house" quality control used three of nine and commercial urine quality control used two of nine MBL. One specification on Q14 was invalid because it included an external quality control in use. Detailed responses stratified by each participating MBL and corresponding questions are presented in Table 3.

**TABLE 2.** Questionnaire of sweat test performance in Croatian medical biochemistry laboratories.

Questions	N/TN
<b>Q1: What is average number of sweat tests per annum in your laboratory?</b>	10/10
a) <50	2
b) 50-100	3
c) 100-300	3
d) >300	3
<b>Q2: Where do you perform sweat stimulation and collection?</b>	10/10
a) in a clinic	4
b) in a laboratory	6
<b>Q3: What method do you use for sweat stimulation?</b>	10/10
a) pilocarpine iontophoresis	10
b) wrapping a patient with plastic material	0
<b>Q4: Who performs sweat stimulation and collection?</b>	10/10
a) laboratory technician and/or technologist	5
b) medical biochemist	1
c) clinical staff	4
<b>Q5: What type of information do you provide to parents or patients about sweat test?</b>	10/10
a) only written	0
b) only verbal	5
c) verbal and written	2
d) by hospital web site	1
e) no information provided	2
<b>Q6: Do you test new-borns less than 2 weeks of age?</b>	10/10
a) yes	2
b) no	8
<b>Q7: Do you test new-borns less than 3 kg of weight?</b>	10/10
a) yes	1
b) no	9

<b>Q8: Number of additional sweat tests carried out after first positive result?</b>	10/10
a) 1	4
b) 2	5
c) > 3	0
<b>Q9: Which electrodes do you use for sweat stimulation?</b>	9/9
a) stainless steel electrodes	0
b) cooper electrodes	3
c) electrodes of flexible size and curvature made by local manufacturer	6
d) electrodes of unspecified origin	0
<b>Q10: How long do you collect sweat after pilocarpine iontophoresis?</b>	9/9
a) < 30 min	1
b) 30 min	8
c) 30 - 40 min	0
d) > 40 min	0
<b>Q11: What is the minimum acceptable sweat weight for chloride analysis?</b>	9/9
a) 50 mg	1
b) 75 mg	5
c) 100 mg	2
d) no criteria for acceptable sweat weight	1
e) other, specify	0
<b>Q12: What do you use for sweat collection?</b>	9/9
a) pre-weighed gauze	7
b) pre-weighed filter paper	2
c) polypropylene or glass tube	0
<b>Q13: What do you do when insufficient sweat weight is collected?</b>	9/10
a) keep testing and carry out chloride analysis	1
b) stop testing and repeat sweat collection another time	7
c) carry out bilateral sweat collection	1
<b>Q14: Do you use internal quality control?</b>	9/9
a) yes, specify	5
b) no	4
<b>Q15: What is the number of sweat test performed annually by the most involved individual staff?</b>	10/10
a) ≤ 10	0
b) 11 - 49	4
c) ≥ 50	5
d) no record	1
<b>Q16: What are your laboratory reference ranges for sweat chloride in infants &lt; 6 months of age?</b>	8/9
a) negative < 30 mmol/L; intermediate 30 - 60 mmol/L; positive > 60 mmol/L	3
b) negative < 60 mmol/L; intermediate 60 - 80 mmol/L; positive > 80 mmol/L	3
c) other, specify	2

<b>Q17: What are your laboratory reference ranges for sweat chloride in infants &gt; 6 months of age and adults?</b>	9/9
a) negative < 40 mmol/L; intermediate 40 - 60 mmol/L; positive > 60 mmol/L	5
b) negative < 60 mmol/L; intermediate 60 - 80 mmol/L; positive > 80 mmol/L	1
c) other, specify	3
<b>Q18: Is collected sweat weight included in the laboratory sweat test report?</b>	9/9
a) yes, specify	3
b) no	6

N – number of responders. TN – total number of participating laboratories.

**TABLE 3.** Stratified responses of medical biochemistry laboratory in national questionnaire.

MBL	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q14a	Q15	Q16	Q17	Q18
1	d	a	a	c	c	b	b	b	c	b	c	a	b	a	A	c	b	a	a
2	c	b	a	a	b	a	a	NR	c	b	b	a	b	a	B	c	b	b	a
3	c	b	a	a	c	b	b	b	b	b	b	b	b	a	B	b	a	a	b
4	b	a	a	c	e	b	b	b	c	a	a	a	b	b	-	b	NR	c	b
5	b	b	a	a	b	b	b	a	c	b	b	a	b	a	B	b	a	a	b
6	a	b	a	b	b	b	b	a	c	b	d	a	NR	b	-	d	c	c	b
7	a	a	a	c	e	b	b	b	c	b	b	a	a	b	-	b	b	a	a
8	d	b	a	a	b	a	b	b	b	b	b	b	b	a	A	c	c	c	b
9	b	a	a	c	d	b	b	a	b	b	c	a	c	b	C	c	a	a	b
10*	c	b	a	a	b	b	b	a	NA	NA	NA	NA	b	NA	NA	c	NA	NA	NA

MBL- medical biochemistry laboratory. Q1 - Q18 – questions defined in Table 2. NR - no response. NA - not applicable. A - urine commercial control. B – “in-house” control. C - external quality control. \*MBL performed sweat conductometry;

## Comments on the survey

Q1, Q2, Q4, Q5, Q15 – Number of 50 sweat tests per annum had been suggested as minimum number of testing for laboratory performing sweat test (19). An annual number of sweat tests more than 50 ensures that involved centre is qualified in this challenging performance. Furthermore, there is a request of minimum testing for individual staff member per annum. In the survey, majority of MBL (8/10) reported more tests per annum that recommended, but two MBL did not meet that request. One of them was in line with other eight MBL regarding number of reported individual staff sweat testing per annum. In four centres (4/10) sweat stimulation and collection was performed in a clinic by clinical staff exclusively. It seems a common practice across Europe that sweat stimu-

lation and collection are settled in a clinic and a laboratory (13,14). Regarding patient information, current guidelines recommend that the centre should provide written or audio/visual material to patients and parents attending the test (8,9). This practice reported three out of ten MBL in the survey. Furthermore, two out of ten centres provided no information to parents and patients about sweat testing.

Q3 – All MBL (10/10) stated the PI as a method for sweat stimulation. It was in accordance with current guidelines that exclusively recognised PI as method of choice (8,9).

Q6, Q7 – Patients suitability address feasibility of their physiological and clinical state for sweat test.

It was reported that patient weight and age had great impact on success of PI (20). Current guidelines discourage sweat testing in new-borns having less than 2 weeks of age and 2-3 kilograms of weight. Majority of surveyed MBL were in accordance with recommendations regarding new-borns age (8/10) and weight (9/10).

Q8 – According to reports in all MBL (10/10) the first positive sweat test result called for one or two more testing for CF diagnosis. It is in accordance with international and national clinical guidelines (8,12). Although sweat test is a gold standard for CF there is common practice of repeating sweat testing in case of the first positive result (9). Sweat test repeating practice makes reported result more confident for CF but it should be always interpreted in context of patient presentation, familiar background genetic testing and new-born screening if the latter is available (8). Although sweat test has superior diagnostic accuracy to genetic testing and equal role in official CF diagnostic pathway in real-life clinical practice retesting patients with initially positive result commonly precede molecular testing (3,12). However positive sweat test may be considered in other clinical conditions not related to CF. Regarding sweat test positive result it is worth to mention that chloride concentration above 160 mmol/L is not linked with human physiology and it is rather an indicator of poor test performance (9).

Q9 – Current guidelines recommend use of stainless steel or copper electrodes for the PI. They should be suitable and fixed size with an extra device that allows stable and safe application on patient flexor surface of forearm (8,9). Six out of nine MBL reported use of electrodes produced by local manufacturer (Figure 1). Those electrodes did not meet guidelines criteria for fixed size due to their design as bracelet of flexible length that is adjustable around patient forearm since size depends of his/her age. Such electrodes design excludes controlled sweat stimulation. It refers particularly to unfixed size of stimulated area and uncontrolled sweat secretion rate that compromises accurate minimum sweat weight acceptable for analysis and increases a possibility of false chloride concentration in report.



**FIGURE 1.** Electrodes for pilocarpine iontophoresis (PI) produced by local manufacturer

Q10 – The sweat collection more than 30 minutes is not supported by guidelines due to fact that the most intensive sweating occurs 10 to 30 minutes after stimulation (8,9). Surveyed MBL declared correct practice in rate 8 out of 9.

Q11, Q13, Q18 – The minimum acceptable sweat weight (MASW) is defined as sweat weight produced by secretion rate  $> 1 \text{ g/m}^2/\text{minute}$  and collected after stimulation (8). MASW is highly depended of stimulation area (electrode and filter/gauze size), sweat secretion rate, collection time and patient suitability including age, weight, race and skin condition (8,9). At low sweat rate  $< 1 \text{ g/m}^2/\text{minute}$ , sweat production is limited contributing to false decrease chloride concentration (21). Correct set-up of MASW is a cornerstone of sweat testing limiting occurrence of false results (9). In the survey, almost all MBL (8/9) reported MASW use on different levels ranging from 50 to 100 mg but five of them used electrodes made by local manufacturer that excluded a rationale for MASW. However most of MBL (7/9) performed the correct action in managing insufficient sweat quantity to stop the test and repeat it another time. An importance of sweat weight for correct interpretation of sweat result was recognised by its inclusion in sweat report (19). Such practice reported 3 out of 9 MBL.

Q12 – The pre-weighed filter paper or gauze are acceptable devices for sweat stimulation and collection if they fit by size and origin (low content of

chloride). Majority of MBL (7/9) preferred pre-weighed gauze over pre-weighed filter paper.

Q14 – Internal quality control (IQC) for sweat test is mandatory part of quality assurance and it is strongly recommended by guidelines. IQC includes commercial and/or “in house” controls (8,9). Five out of nine MBL introduced IQC as part of quality assessment. The types of IQC in use were “in house” controls or commercial urine controls. Chloride concentrations levels in commercial urine controls typically range from 50 to 150 mmol/L and correspond to chloride intermediate to high concentrations in sweat but low levels (30 - 40 mmol/L) important for clinical decision remained out of quality assessment.

Q16, Q17 – Current guidelines recommend two levels of sweat chloride reference ranges depending on patient’s age. Only 3 out of 8 MBL stated correct reference ranges for infants aged 6 months or less while results of reported use of reference ranges for older infants and adults were better and included 5 out of 8 MBL.

## Discussion

Sweat chloride measurement is a part of schemes of several external quality assessment providers (10,22,23). EQA, together with internal quality control (IQC) is an optimal tool for monitoring analytical phase of testing. Although all of them must be part of sweat test performance in clinical laboratories, our results reveal lack of internal quality assessment in some Croatian MBL and previously published Italian sweat test survey stressed low access of laboratories in external quality assessment (13). One of possible obstacles for Croatian MBL may be limited selection of methods for evaluation offered by providers of commercial quality controls nevertheless an “in-house” IQC is allowed by current guidelines. Listed selection mainly includes coulometry or ISE, but almost all Croatian MBL offer mercurimetric titration. According to guidelines it is acceptable method for sweat chloride analysis, but it should be verified to insure reliability of reported results. In the field of laboratory medicine, verification process commonly follows standardisation. Lack of standardisation is main

drawback in 2015 CROQALM exercises presenting poor chloride measurement of involved MBL. It was made up with introduction of uniform protocol resulting in better data obtained in following exercises. Such standard less practice may compromise quality of testing and have negative impact on clinical decision. External quality control assessment is an optimal way to find out inconsistencies in testing and making corrections as was proved in our study. Furthermore, it is also an educational process with the final goal to permanently improve testing in clinical laboratories (24).

External quality control focused only on sweat chloride measurement gives an insight to the analytical phase of the testing but excludes an assessment of sweat stimulation, collection and reporting results. Common way to check up on those issues is use of comprehensive questionnaire based on current sweat test performance guidelines.

A design of presented questionnaire was partially made in accordance to outdated UK guideline from 2003 (19). It is particularly addressed to Q1 and Q18. Authors believed that an annual number of sweat testing (Q1) may give a clear picture of sweat test workload in one MBL as well as inclusion of sweat weight on report (Q18) may provide an extra information about accuracy of sweat test performance. Therefore, questionnaire also included all relevant topics from updated UK and CLSI guidelines (8,9). In addition, it brings up some potentially useful data about use of unverified devices and raises the concern about accuracy of PI performance in Croatian MBL. From presented data low adherence to current guidelines in areas of patient information, PI performance, quality assessment and report of sweat chloride reference ranges are evident. The latter is extremely important for proper clinical decision and evaluation of results.

However, it seems that inconsistencies of different origin are common practice in real-life sweat testing across the globe. Our study is in line with recently published studies that revealed several areas of concerns ranging from patients’ information, internal and external quality assessment to the use of appropriate reference ranges despite of internationally accepted guidelines that made a path to standardised performance of sweat test (13,14,25).

In conclusion, results of this survey pointed out a need for standardisation of sweat test practice in Croatian MBL.

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## Potential conflicts of interest

None declared.

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