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The Italian External Quality Assessment Program for CF Sweat Chloride Test: Results of the 2015 Round

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Abstract

Background: Sweat chloride test is the gold standard test for cystic fibrosis (CF) diagnosis. In 2014 the Istituto Superiore di Sanità (ISS) established the first Italian pilot external quality assessment (EQA) program for CF sweat chloride test. In 2015 this activity was recognized as a third party service carried out by the ISS. The present paper describes the results of the first official 2015 sweat chloride test EQA program and results are compared with the 2014 round ones. ***Methods:*** the scheme is prospective; participation is open to Italian laboratories performing sweat test analysis for CF diagnosis. Enrollment is voluntary and since 2015 the payment of a fee is required. Participants are registered identified by an identification number known only to the ISS. Assessment covers analysis, interpretation and reporting. ***Results:*** thirteen laboratories, belonging to the Italian public Referral Centers for CF, participated in the 2015 round; nine already participated in 2014. Variability in scores of chloride titration and heterogeneity in interpretation / reporting results were identified in 2014 and 2015. ***Conclusions:*** results show variability in performance of laboratories indicating that the quality of laboratory performance is unpredictable unless EQA participation is mandatory as a component of compulsory laboratory accreditation..

Keywords: Sweat chloride test, Liver disease; Laboratory methods; Quality assurance & control

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1. Introduction

Diagnostic testing in CF is based on the sweat chloride test (SCT) in the context of appropriate signs and symptoms of disease and results of the cystic fibrosis transmembrane regulator (CFTR) protein mutation analysis. The SCT is a well-established functional assessment of CFTR that has been available for decades to diagnose CF (Gibson LE, 1959) and, recently, to test the effect of CFTR potentiators (Accurso et al., 2014) Italian recommendations for appropriate execution and interpretation of sweat test (Gruppo di Lavoro della Società Italiana per lo Studio della Fibrosi Cistica, Il test del sudore. 2007) suggest how to correctly perform analyses and interpret results.

Sweat testing is currently performed in approximately 37 laboratories across Italy belonging to the Italian public Referral Centers and in many other laboratories not belonging to such Centers. However the absence of any updated census doesn't allow knowing exactly the total number of laboratories performing SCT nationwide.

It is of critical importance that SCT is carried out accurately with measurement of relevant analytes to allow clinical interpretation of results. Italian audit performed in 2008 showed areas of inconsistencies in current practices for SCT, highlighting the needs of national guidelines to improve practice and management strategies (Cirilli et al, 2008; Cirilli et al, 2008). In order to increase and monitor quality in laboratory performing SCT, an EQA SCT pilot program was performed in 2014 at the Italian National Centre for Rare Diseases (CNMR) of the Istituto Superiore di Sanità (ISS, Rome); in 2015 the activity was recognized as institutional through the publication of Official Bulletin of the Italian Republic (Official Bulletin of the Italian Republic, 2015; Salvatore et al, 2016) and the SCT EQA program is now permanent. Aim of this study is to report results of the first official 2015 sweat chloride test EQA program compared with the 2014 round ones.

2. Methods

			LABORATORIES															
			a	b*	c	d	e*	f	g*	h	i	j	k	l	m	min score	mean score	max score
Qualitative description of the structures																		
Number of test/year			10	10	10	10	10	10	10	10	10	10	10	10	0	0	9,2	10
Number of test/year/technician			10	10	10	10	10	10	10	10	10	10	10	10	10	10	10,0	10
Time of test execution			10	10	10	8	10	8	10	10	10	0	10	8	10	0	8,8	10
Stimulation method			10	10	10	10	10	10	10	10	10	0	10	10	10	0	9,2	10
Sweat collection			10	10	10	10	10	10	10	10	10	10	10	10	10	10	10,0	10
Analytical method			10	10	10	10	10	10	10	10	0	10	10	10	10	0	9,2	10
Quantitative analytical performance																		
Sample SLS-1 (Reference value: 20,25 mEq/L; Cl^- mean value: 19,76 mEq/L; expected correct interpretation: "unlikely CF")																		
reporting information			10,0	9,5	10,0	10,0	8,0	10,0	7,5	8,5	9,5	3,5	9,5	10,0	10,0	3,5	8,9	10,0
chloride concentration value			8,1	9,8	0,7	0,0	0,0	0,0	0,0	3,2	5,7	8,1	4,4	5,7	2,5	0,0	3,7	9,8
clinical sensitivity			10,0	10,0	10,0	10,0	10,0	10,0	0,0	10,0	10,0	10,0	10,0	10,0	10,0	0,0	9,2	10,0
Sample SLS-2 (Reference value: 39,53 mEq/L; Cl^- mean value: 41,76 mEq/L; expected correct interpretation: "intermediate result which requires further cystic fibrosis assessment")																		
reporting information			10,0	9,5	10,0	10,0	8,0	10,0	6,0	8,5	8,0	3,5	10,0	10,0	10,0	3,5	8,7	10,0
chloride concentration value			9,3	6,9	0,0	9,2	0,0	0,0	0,0	7,9	0,7	7,9	9,2	0,0	3,7	0,0	4,2	9,3
clinical sensitivity			10,0	10,0	10,0	10,0	0,0	10,0	0,0	10,0	10,0	10,0	10,0	10,0	10,0	0,0	8,5	10,0
Sample SLS-3 (Reference value: 200,00 mEq/L; Cl^- mean value: 195,95 mEq/L; expected correct interpretation: "non-physiological value: results should be questioned")																		
reporting information			10,0	9,5	10,0	10,0	7,0	10,0	6,00	8,5	9,5	3,5	10,0	10,0	10,0	3,5	8,7	10,0
chloride concentration value			7,4	3,7	7,4	6,8	9,7	8,0	1,2	9,1	1,9	8,0	5,8	0,9	8,2	1,2	6,0	9,7
clinical sensitivity			10,0	0,0	10,0	0,0	0,0	10,0	0,0	0,0	10	0,0	0,0	0,0	0,0	0,0	3,1	10,0
TOTAL MAX SCORE FOR ALL CATEGORIES = 150			145	129	128	124	103	126	81	126	115	95	129	115	114	81	118	145
Laboratories in dark grey participated in 2014 and 2015 IEQA-ST rounds; laboratories in light grey participated to 2015 IEQA-ST round. * Laboratories obtaining a critical score in choloride titration in 2014 and 2015																		

Table 1: IEQ-TS 2015 general results

The pilot IEQA-SCT has been fully described by Salvatore et al. (Salvatore et al., 2016). Overall, ten and thirteen laboratories, included among the 37 Italian CF public Referral Centers, participated in 2014 and 2015 respectively.

It's not possible to state the percentage of the Italian laboratories performing SCT and participating in the IEQA-SCT program since there is no updated and available census at the moment. Participation is voluntary. An Identification Number (ID) was assigned to each laboratory by the scheme organizer (ISS). In the present paper laboratories are identified by a progressive alphabetical ID from *a* to *m*.

Nine laboratories out of ten enrolled in 2014 (*a* to *i*) participated to both rounds; laboratories *j* to *m* participated to 2015 round only. Each laboratory received three commercial different sweat-like samples (SLS), validated by ISS, consisting in aqueous material miming normal sweat composition (Table 1).

Expected chloride concentration of samples in 2015 is comparable to 2014 ones; as regard sample SLS-1 (20 mEq/L) in 2014 the mock weight of sweat collected (indicated by providers) was not sufficient to perform analysis (less than 75 mg) and laboratory should have indicated it in interpretation of results. Each sample had specific mock clinical indications and had to be analyzed according to routine procedures. Assessment, performed by a panel of experts, covered analytical performance, interpretation and reporting of results and was based on the Italian guidelines (Gruppo di Lavoro della Società Italiana per lo Studio della Fibrosi Cistica, Il test del sudore. 2007).

Table 2 summarizes assessment criteria and scores assigned for each parameter. Overall two main categories were taken into account: *qualitative description of the laboratories* and *quantitative analytical performance per sample* (Table 2). Information about qualitative description of the laboratories (i.e. contact information; accreditation/certification; number of sweat tests/year/technician; failure rate; method of stimulation; collection and analysis) were referred to 2014 data and collected through reports and on-line pre-test questionnaire given to all participants.

Qualitative description of the structures			
	Score assigned in IEQA-SCT		
Number of test/year *	10 (> than 200 tests per year)		0 (< than 200 tests per year)
Number of test/year/technician *	10(> than 50 tests per year)		0 (< than 50 test per year)
Time of test execution *	10 (≤ 24 hrs)	8 (> 24 hrs and < than 48 hrs)	0 (72 hrs or more)
Stimulation method *	10 (if by pilocarpine nitrate)		0 (other methods)
Sweat collection *	10 (if onto filter paper)		0 (other)
Analytical method *	10 (if by coulometry, colorimetry, ISEs)		0 (other methods)
Quantitative Analytical performance			
Reporting information* (at least two patient identification data; date of test; date of primary sample collection; date of report; weight and volume of sweat collected; indication of insufficient collection (< 75mg); stimulation method; analyte(s); analytical method; reference intervals (normal results if < 40 mEq/L or < 30 mEq/l in patients more and less than 6 months of age respectively; intermediate results if 40-60 mEq/L or when the patients is less than 6 months of age, 30-60 mEq/L; diagnosis of CF if > 60 mEq/L); interpretation of results; presence or not of report signature; report clear legibility)	10 (if complete and correct)		0-9 (if not complete and or not correct)
Chloride concentration value	0 to 10 Values exceeding the 75% of the expected ones were not included in the analyses (i.e. for an expected 20 mEq/L Cl^- concentration value, all values reported as more than 35 mEq/L were not included in the following calculation). A 20% of error was accepted for an expected value of 20 mEq/L; 10% of error was accepted for an expected value of 100 mEq/L. A proportional % of error was considered for all the values between 20 and 100 mEq/L. For each sample, mean Cl^- value was calculated as the mean of all the measurements from laboratories.		
Clinical sensitivity	10 (correct)		0 (not correct)

*Assessment criteria based on Italian Guidelines

Table 2: assessment criteria and scores assigned

All data were managed through a web utility (Salvatore et al, 2016). All results were normalized (maximum score = 150) in order to make a comparison between 2014 and 2015 data. Scores were assigned by a panel of experts, composed by representatives of CNMR-ISS and National Societies (Italian Cystic Fibrosis Society, SIFC; Italian Society for the Study of Inborn Metabolic Diseases and Newborn Screening, SIMMESN; Italian Society of Clinical Biochemistry and Clinical Molecular Biology, SIBiOC), both online and collegially in a dedicated meeting.

3. Results

In 2015, 13 laboratories participated in the first official round of IEQA-SCT and nine of them participated also in the 2014 round (laboratories *a* to *i*). A great variability between 2014 and 2015 was identified for scores relative to chloride titration (*median score value = 15,73/30,* in 2014 and *13,93/30* in 2015) and clinical sensitivity (median score = *26/30* in 2014 and *20,76/30* in 2015). Median scores were 117,4/150 and 117,6/150 in 2014 and 2015 respectively; scores ranged from 45,3/150 (min value) to 139/150 (max value) in 2014 and 80,67/150 (min value) to 144,82/150 in 2015 (Figure 1).

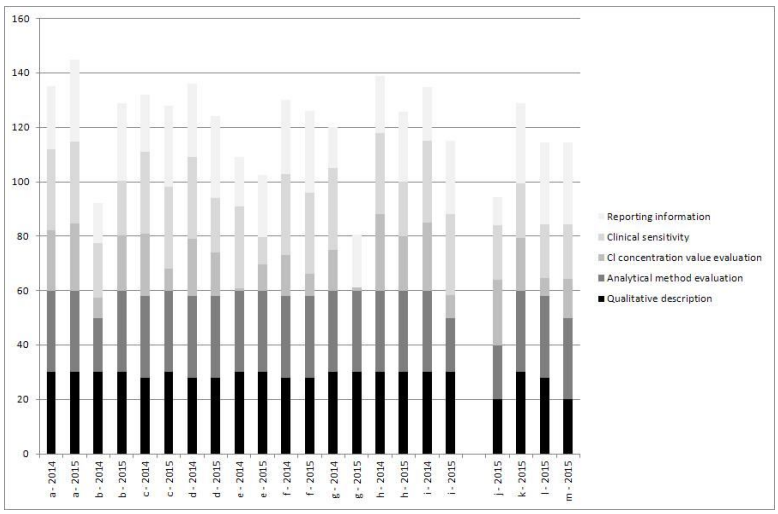

Figure 1: summary of results relative to all samples per laboratory. On the x axis laboratories participating in the 2014 and 2015 round (*a* to *i*) and in 2015 (*j* to *m*); on the y axis reports scores.

Qualitative description of the laboratories

All laboratories, except one (laboratory *i*), participating in both experiences reached an optimum score ranking from 8/10 to 10/10 (Table 1). Amongst laboratories which participated for the first time in 2015, laboratory *j* had an insufficient score in "time of test execution" and in "stimulation method" (not reported to IEQA-SCT provider); laboratory *m* obtained an insufficient score in "number of test per year" (Gruppo di Lavoro della Società Italiana per lo Studio della Fibrosi Cistica, Il test del sudore, 2007).

Quantitative analytical performance

SLS-1 (Reference value 20,00 mEq/L, quantity of sweat collected = 55mg ie insufficient in 2014 and 20,25 mEq/L 2015): chloride concentration results ranked from 0/10 to 9,8/10 (mean score = 3,7/10); laboratories *e* and *g* obtained a critical score in 2015 (0/10) and 2014 (ranking score 0,7/10 and 0,8/10 respectively). Laboratories *c*, *d* and *f* obtained a 0/10 score in 2015 while in 2014 the score assigned ranked between 5,6/10 and 7,3/10. On the other hand, laboratory *b* had a good score in 2015 (score = 9,8/10) while in 2014 obtained a 0/10 score.

As regards clinical sensitivity (consistency of a sweat chloride result normal range), all laboratories except one (laboratory *g*, score 0/0) interpreted correctly the results. Error by laboratory *g* was due to a not appropriate use of adopted reference intervals. Laboratory *g* worsened its performance from 2014 to 2015 (from a 10/10 to a 0/10 score respectively); on the other hand, laboratory *b* improved its performance from 0/0 to 10/10.

SLS-2 (Reference value 40,00 mEq/L, in 2014 and 39,53 mEq/L 2015): chloride concentration results ranked from 0/10 to 9,3/10 (mean score = 4,2/10). Six laboratories (namely *c, e, f, g, i, l*) out of 13 (46,15%) made a wrong measurement in chloride titration (ranking score from 0/10 to 0,7/10). Laboratory *e* obtained a critical score also in 2014 (score 0/10); laboratories *c, f, g* and *i* worsened their performance from 2014 (score ranking from 5/10 to 9,6/10) to 2015 (score ranking from 0/10 to 0,7/10). As regards clinical sensitivity, two laboratories (*e* and *g*) obtained a critical score in interpretation in 2015. A poor score was assigned to laboratory *e* because the interpretation was missing; laboratory *g* made a wrong interpretation of the result due to a not appropriate use of adopted reference intervals. Both laboratories worsened their performance from 2014 to 2015 (from 10 to 0 score).

SLS-3 (Reference value 90,00 mEq/L, in 2014 and 200,00 mEq/L 2015): chloride concentration results ranked from 0,9/10 to 9,7/10 (mean score = 6,0/10). Only laboratory *l* made a wrong chloride titration (0,9/10). Laboratory *e* improved its performance from 2014 to 2015 (from 0 to 9,7/10 score). Nine laboratories out of 13 (69, 23%) obtained a critical score in clinical sensitivity as they didn't report that the chloride value was not physiological and consequently the result should have been questioned. Laboratories *b, d, e, g* and *h* worsened their performance from 2014 to 2015 (from 10/10 to 0/10 score).

Reports assessment

Almost all reports were incomplete, missing information concerning one or more parameters. Scores ranged from 3,5/10 to 10/10 and most frequently missing information concerned "interpretation of results" (15,4%, in sample SLS-1; 30,8% in sample SLS-2 and 15,4% sample SLS-3), "reference intervals" (23,0%, in SLS-1; 30,8% in SLS-2 and 15,4% in SLS-3), and "date of primary sample collection" (15,4%, in SLS-1; 15,4% in SLS-2 and 23,1% in SLS-3). No difference was detected among laboratories participating both in 2014 and 2015.

4. Conclusions

Sweat chloride test is the gold standard to diagnose CF and to monitor patients during molecular therapies, where accurate results are required. Recent papers identify different issues about laboratories standardization in execution, interpretation and reporting of results (Cirilli et al, 2008; Cirilli et al, 2012; LeGrys 2000; LeGrys 2001; Kirk JM, 2000). The first Italian national program on external quality assessment for CF SCT was piloted in 2014; in 2015 this activity was recognized as a third party service carried out by the ISS (Official Bulletin of the Italian Republic, 2015), therefore the first official round started and was completed.

A total of fourteen laboratories were monitored all over the two years period: nine (out of 10 enrolled in 2014) participated in both EQA schemes, four in the 2015 round. It is not possible to state the exact percentage of laboratories performing SCT and participating in the IEQA-SCT, since there is no updated census available; however, we can say that about 30% of Italian laboratories performing CF SCT, belonging to the public cystic fibrosis centers, are currently monitored. Even though a general good level of quality was identified in the *qualitative description of the structures* to which laboratories belong, *quantitative analytical performance* was characterized by a significant heterogeneity.

In particular, there was variability in scores as regards the evaluation of chloride titration both in 2014 and in 2015; notably, most errors in Cl^- titration were made by six different laboratories participating in both 2014 and 2015 rounds. Currently we cannot exclude that errors are due to methodological, equipment or technical problems caused by the unskilled personnel (Miller WG et al, 2011).

One single laboratory, participating in both rounds, improved its performance from 2014 to 2015. As regards clinical sensitivity, six different laboratories participating in both 2014 and 2015 rounds obtained a poor score. In particular, in 2015 about 70% of laboratories failed to make suggestions when the chloride value was reported as not physiological (sample SLS3) as clearly indicated by National Guidelines (Gruppo di Lavoro della Società Italiana per lo Studio della Fibrosi Cistica, Il test del sudore. 2007).

Heterogeneity was observed in the modality of results reporting. Most frequently missing information concerned "reference intervals", "date of primary sample collection" and in particular "interpretation" that affected clinical sensitivity as previously discussed.

Variability in results also indicates that until EQA participation becomes mandatory as a component of compulsory laboratory accreditation, the quality of laboratory performance is unpredictable (Hastings et al, 2008). Moreover, it is noteworthy to underline that two laboratories making a wrong chloride titration in one or more samples in the 2014 pilot scheme didn't address the internal analytical problem and performed poorly also in the 2015 round for two samples. In this respect, it is generally a good idea for laboratories to have well-written standard operating procedures that are based on published guidelines; these should, along with training, address the issues of execution, interpretation and reporting of results.

Currently we are harmonizing the activity of this program with existing Italian and European EQA schemes and improving the structure of the program by a new marking system when two categories of performance are defined, i.e. poor and satisfactory. Moreover, it would be desirable to combine a quality control program to an implementation plan of quality improvement.

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