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Italian external quality assessment program for cystic fibrosis sweat chloride test: a 2015 and 2016 results comparison

Marco Salvatore[1], Giovanna Floridia[2], Annalisa Amato[1], Federica Censi[1], Maria Chiara de Stefano[1], Gianluca Ferrari[1], Fabrizio Tosto[1], Domenica Taruscio[1] and Italian EQA-SCT Working Group*

[1]*Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, Rome, Italy*
[2]*Unità di Bioetica, Istituto Superiore di Sanità, Rome, Italy*
**The members of the Italian EQA-SCT Working Group are listed before the References*

Abstract

Background. Diagnostic testing in cystic fibrosis (CF) is based on the sweat chloride test (SCT) in the context of appropriate signs and symptoms of disease and results of the gene mutation analysis. In 2014 the Istituto Superiore di Sanità (ISS) established a pilot Italian external quality assessment program for CF sweat chloride test (Italian EQA-SCT). In 2015 this activity was recognized as a third party service carried out by the ISS. The aim of the paper is to compare 2015 and 2016 results and experiences.

Methods. The scheme is prospective; enrollment is voluntary and the payment of a fee is required. Participants are registered and identified by a specific Identification Number (ID) through a dedicated web-facility. Assessment covers analysis, interpretation and reporting of results.

Results. Thirteen and fifteen laboratories, participated in the 2015 and 2016 round respectively. Seven laboratories participated constantly from 2014, eleven participated both in 2015 and 2016 and four participated in 2016 for the first time. Variability in scores of chloride titration and heterogeneity in interpretation/reporting results were detected in both rounds. A total of 18 critical errors in chloride titration were made by eight different participants. Four laboratories made errors in chloride titration in 2015 but drastically improved their performance in 2016. In 2016 poor performance criteria were established and adopted.

Conclusions. Even though results show variability in performance of laboratories, constant and mandatory participation may contribute to the improvement of performance and quality reached by laboratory.

Key words

- sweat chloride test
- liver disease
- laboratory methods
- quality assurance control

INTRODUCTION

Cystic fibrosis (CF, OMIM 219700) is a life-limiting autosomal recessive disorder that affects ~70 000 individuals worldwide. The condition affects primarily those of European descent, although cystic fibrosis has been reported in all ethnicities. Twenty-eight years ago, a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene was found to be the most common cause of CF. Identification of disease-causing variants in CFTR contributed a tool for both the diagnosis of cystic fibrosis and the identification of CF carriers, demonstrated the degree to which CFTR dysfunction correlates with clinical features. The identification of more than 2000 CFTR mutations/variants, either CF causing or with variable clinical consequences, and genotype-phenotype studies revealed that CFTR dysfunction due to several variants can create different phenotypes in the cystic fibrosis disease spectrum. Over the past few years, there has been remarkable progress in the development of small-molecule therapy targeting CFTR bearing select disease-causing variants as correctors and/or potentiators of the protein [1].

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 CFTR gene mutation analysis. The SCT is a well-established functional assessment of CFTR that has been available for decades to diagnose CF [2] and, recently, to test the effect of CFTR modifier therapies [3]. A general significant correlation between the SCT and clinical manifestations

Address for correspondence: Marco Salvatore, Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: marco.salvatore@iss.it.

has been recently described; however, the same genotype can give rise to a wide range of sweat test values and highly similar sweat test values may be observed in populations with different clinical manifestations [4].

Italian recommendations for appropriate execution and interpretation of sweat test suggest how to correctly perform analyses and interpret results [5].

The precise number of Italian laboratories performing sweat test nationwide is actually not known since there is not any updated census available; however we know for sure that 37 laboratories, belonging to the Italian Referral Care Centres for Cystic Fibrosis, perform SCT.

It is of critical importance that SCT is carried out accurately with measurement of relevant analytes to allow clinical interpretation of results. Two Italian audits performed in 2006 and repeated in 2009 showed areas of inconsistencies in current practices for SCT, highlighting the needs of national initiatives aimed to improve the general performance of sweat test [6, 7]. In order to increase and monitor quality in laboratories performing SCT, an Italian 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 and therefore permanent as reported in the Official Bulletin of the Italian Republic [8-10]. In 2016 poor performance criteria were introduced. Aim of this study is to compare results of the 2015 and 2016 EQA-SCT rounds.

MATERIALS AND METHODS

The pilot Italian EQA-SCT has been fully described by Salvatore *et al*. [9, 10]. The program is performed once per year. Overall thirteen and fifteen laboratories, among the 37 Italian CF public Referral Care Centers, participated in 2015 and 2016 respectively. It is not possible to state the total number of Italian laboratories performing SCT and the percentage participating to the Italian EQA-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 *q*.

Seven laboratories participated constantly to Italian EQA-SCT from 2014 (pilot experience [9, 10], namely laboratories *a, c, e, f, g, h, i*); eleven participated to both rounds (*a, c, e, f, g, h, i, j, k, l, m*) and four participated in 2016 for the first time (laboratories *n, o, p, q*). Laboratories *b* and *d* participated in 2015 round but not in the 2016 one.

Assessment

A panel of experts, composed by representatives from both ISS and National Scientific Societies involved in cystic fibrosis research, clinics and management (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), assessed all the results, both online (by using a dedicated web-facility) and collegially in *de visu* meetings organized by the ISS. All data were treated anonymously and the identity of each laboratory was unknown to the assessors.

Assessment covered analytical performance, interpretation and reporting of results according to the Italian guidelines [5]. *Table 1* summarizes assessment criteria and scores assigned for each parameter in 2015 and 2016.

The *qualitative description of the laboratories* was assessed in the pilot and in the 2015 round through reports and an *ad hoc* online pre-test questionnaire administered to all participants [9].

In 2015 and in 2016 two main categories were taken into account and are discussed in the present paper: a) *technical adequacy in performing sweat chloride test* that includes stimulation method, sweat collection, analytical method; b) *quantitative analytical performance per sample, i.e.* reporting information, chloride concentration value, clinical sensitivity *(Table 1)*.

Reporting information assessment consisted in the evaluation of the correctness and completeness of the following criteria: patient identification data; date of test; date of sample collection; date of report; weight and volume of sweat collected; indication of insufficient collection (< 75mg); stimulation method; analyte(s); analytical method; chloride reference intervals (normal results if < 40 mEq/L; < 30 mEq/L in subjects less than 6 months of age; intermediate results if 40-60 mEq/L, 30-60 mEq/L insubjects less than 6 months of age, abnormal if > 60 mEq/L); interpretation of results; presence of report signature; report clear legibility.

Clinical sensitivity was defined as the consistency of a sweat chloride result with the correct range and the clinical interpretation of the result.

In 2016 Italian EQA-SCT poor performance criteria were also established and poor performance was assigned to laboratories when *i)* exceeding more than 50% the reference values in chloride concentration titration, or *ii)* getting titration value(s) wrong because of unintentional sample exchange and/or clerical or transcription errors, or *iii)* submitting a report where the results interpretation was missing or wrong and/or *iv)* submitting reports where fundamental information was missing.

Samples

Three sweat-like-samples (SLS) were commercially prepared (LTA s.r.l., Milano, Italy) and consisted of an aqueous material mining the normal sweat composition; in particular they contained: dipotassium carbonate, lactic acid, carbamide, glucose, albumin, thimerosal, demineralised water and NaCl (this latter at established range concentrations).

Two independent ISS Working Units quantified and validated all samples before dispatch performing a minimum of 40 measures/sample; the reference value was defined as the mean of all measures. The samples dispatch of 0.5 mL per aliquot, labeled with specific identification codes (namely "Sample I-EQA-SLS-1", "Sample I-EQA-SLS-2" and "Sample I-EQA-SLS-3"; *Table 2*), was single and performed once per year at

Table 1
Assessment criteria and scores assigned

Technical adequacy in performing sweat chloride test (2015 and 2016)			
Stimulation method*	**10** (2015) **3.3** (2016) if by pilocarpine nitrate		**0** (2015) **0** (2016) other methods
Sweat collection *	**10** (2015) **3.3** (2016) if onto filter paper		**0** (2015) **0** (2016) other methods
Analytical method *	**10** (2015) **3.3** (2016) if by coulometry, colorimetry, ISEs		**0** (2015) **0** (2016) other methods
Quantitative analytical performance (2015 and 2016)			
Reporting information* (patient identification data; date of test; date of sample collection; date of report; weight and volume of sweat collected; indication of insufficient collection (< 75mg); stimulation method; analyte(s); analytical method; chloride reference intervals (normal results if < 40 mEq/L; < 30 mEq/L in subjects less than 6 months of age; intermediate results if 40-60 mEq/L, 30-60 mEq/L insubjects less than 6 months of age; abnormal if > 60 mEq/L); interpretation of results; presence of report signature; report clear legibility)	**10** *(per sample)* if complete and correct		**0 to 9** *(per sample)* if not complete and or not correct
Chloride concentration value	0 to 10 *(per sample)* Values exceeding the 75% (*50% in 2016*) of the reference values 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.		
Clinical sensitivity	**10** *(per sample)* correct		**0** *(per sample)* not correct
Qualitative description of the structures (assessed only in 2015 round)			
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)

*Assessment based on the Italian Sweat Test Guidelines [5]

room temperature; mock clinical information together with technical data was provided (*Table 2*) [9, 10]. Laboratories had to analyze samples according to their routine procedures.

Data management and results elaboration

All data were managed through a dedicated web-facility [9, 10] and, in order to compare 2015 and 2016 data, results concerning "technical adequacy in performing sweat chloride test" were normalized (total maximum score = 10) and those ones relative to "qualitative description of the structure" were not taken into account as mentioned above in the "assessment" section *(Table 2* and *Table 3)*.

RESULTS

Thirteen laboratories participated in the 2015 Italian EQA-SCT and 15 in 2016. Eleven participated to both rounds; two laboratories participated only in 2015 (laboratory *b* and *d*) and 4 only in 2016 (laboratory *n* to *p*).

A great variability between 2015 and 2016 was observed for chloride concentration and clinical sensitivity. Scores, related to the assessment of all parameters, ranged from 30.6/100 to 94.7/100 in 2015 (median score = 70.5/100) *(Figure 1* and *Table 1)* and 20/100 to 100/100 in 2016 (median score = 81.3/100) *(Figure 1* and *Table 2)*.

Score per parameter is described in *Figure 1*; "technical adequacy in performing sweat chloride test" results were satisfactory in both rounds.

A significant variability in evaluation of chloride titration was instead observed both in 2015 and in 2016 *(Figure 1; Table 1* and *2)*; a total of 18 critical errors in chloride titration (12 errors in 2015 and 6 errors in 2016; score range = 0 to 0.9) were made by eight different participating laboratories (namely *a, f, g, i, j, k, m, p*). Laboratories *f, g* and *k* participated to both 2015 and 2016 schemes. Laboratories *a, j, i,* and *m* made errors in chloride titration samples in 2015 but drastically improved their performance in 2016 and their score changed from 8.3/30 to 20.4/30 for laboratory *a*, 8,1/30 to 19,7/30 for laboratory *i*; 16/30 to 30/30 for labora-

Table 2
Samples mock clinical and technical information provided to participating laboratories in 2015 and 2016 I-EQA-SCT program. Mean Cl^- value is the median of all the measurements from laboratories

Sample		Mock clinical information	Sweat collected (information provided by EQA provider) (mg)	Reference value (mEq/L)	Chloride concentration mean value (mEq/L)	Expected correct interpretation
I-EQA-SLS-1	*2015*	Three years old child with recurrent bronchial pneumonia and chronic cough.	102	20.25	19.76	"unlikely CF"
	2016	Thirty days aged female identified as part of a screening programme with raised IRT and carrier of a CF-causing mutation.	86	35.66	35.10	"intermediate result which requires further cystic fibrosis assessment"
I-EQA-SLS-2	*2015*	Two months old asymptomatic female positive to CF neonatal screening.	80	39.53	41.76	"intermediate result which requires further cystic fibrosis assessment"
	2016	Sixty-two aged male, grandfather of a female child with two CF-causing mutations. The patient has chronic bronchopneumopathy and cirrhosis.	120	54.96	56.80	"intermediate result which requires further cystic fibrosis assessment"
I-EQA-SLS-3	*2015*	Sixteen years old boy with inadequate weight gain and recurrent respiratory infections.	130	200.00	195.95	"non-physiological value: results should be questioned"
	2016	Child with infection-induced wheezing, negative neonatal screening, two acute pancreatitis episodes and pancreas divisum diagnosis.	92	100.00	115.45	"supports a diagnosis of cystic fibrosis"

tory *j* and 9.7/30 to 21.3/30 for laboratory *m (Figure 1)*. Laboratory *p* was a first time participating laboratory and wrong chloride titration of all the three samples in 2016.

Technical adequacy in performing sweat chloride test

Technical adequacy in performing sweat chloride test included stimulation method, sweat collection and analytical method assessment. Both in 2015 and 2016 almost all laboratories reach the highest score (10/10). In 2015 laboratories *a* and *d* obtained a "0" score in "analytical method" and "stimulation method" assessment respectively since these information were not reported in their reports *(Table 3* and *Table 4)*.

Quantitative analytical performance

SLS-1

2015: Reference value 20.25 mEq/L; Mean value: 19.76 mEq/L; interpretation: "unlikely CF"

2016: Reference value 35.66 mEq/L; Mean value: 35.10 mEq/L; interpretation: "intermediate result which requires further cystic fibrosis assessment"

In 2015 chloride concentration results ranked from 0/10 to 9.8/10 (mean score = 3.7/10); five laboratories (*f, i, j, k* and *m*) got a wrong chloride titration and were marked with 0 to 0.7 score *(Table 3)*.

In 2016 chloride concentration results ranked from 0/10 to 10/10 (mean score = 7.9); laboratories *f* (participating also to 2015 round) and *p* (participating in 2016 for the first time) were marked with a 0 score *(Table 4)*.

Clinical sensitivity results in 2015 were good for almost all laboratories except for laboratory *k* (score 0/0); error was due to a not appropriate use of adopted reference intervals.

In 2016 three laboratories reported a wrong interpretation of results: laboratory *k* was marked with a 0 score since interpretation of results was not present in the report sent to providers. Also laboratories *p* and *q* (first time participating) didn't include an interpretation of results in their reports and were both marked with 0 score.

Of note, clinical sensitivity of sample SLS-1 is not comparable between 2015 and 2016; in 2015 interpretation was referred to an *"unlikely CF"* and in 2016 to an *"intermediate result which requires further cystic fibrosis assessment"*; this may be reflected on the better results obtained in the first round (2015).

SLS-2

2015: Reference value 39.53 mEq/L; Mean value: 4176 mEq/L; interpretation: "intermediate result which requires further cystic fibrosis assessment"

2016: Reference value 5496 mEq/L; Mean value: 5680 mEq/L; interpretation: "intermediate result which requires further cystic fibrosis assessment"

Chloride concentration results ranked from 0/10 to 9.3/10 (mean score = 4.2/10). Six laboratories (namely *a, e, f, i, k, m*) out of 13 (46.15%) made a wrong measurement in chloride titration (ranking score from 0/10 to 0.7/10).

Table 3
Italian EQA-SCT 2015 general results

	PARTICIPANTS IN 2015 Italian EQA-SCT ROUND															
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*min score*	*mean score*	*max score*
Technical adequacy in performing sweat chloride test																
Stimulation method	3.3	3.3	3.3	0.0	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	**0.0**	**3.0**	**3.3**
Sweat collection	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	**3.3**	**3.3**	**3.3**
Analytical method	0.0	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	**0.0**	**3.0**	**3.3**
min score	**0.0**	**3.3**	**3.3**	**0.0**	**3.3**	**3.3**	**3.3**	**3.3**	**0.0**	**0.0**	**3.3**	**3.3**	**3.3**			
mean score	**2.2**	**3.3**	**3.3**	**2.2**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**			
max score	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**			
Quantitative analytical performance																
• **Sample SLS-1.** *Reference Cl- value: 20.25 mEq/L; expected interpretation: "unlikely CF"*																
Reporting information	9.5	10.0	9.5	3.5	9.5	10.0	10.0	10.0	10.0	10.0	7.5	8.5	8.0	**3.5**	**8.9**	**10**
Chloride concentration value	5.7	8.1	9.8	8.1	4.4	0.0	5.7	2.5	0.7	0.0	0.0	3.2	0.0	**0**	**3.7**	**9.8**
Clinical sensitivity	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	0.0	10.0	10.0	**0**	**9.2**	**10**
min score	**5.7**	**8.1**	**9.5**	**3.5**	**4.4**	**0**	**5.7**	**2.5**	**0.7**	**0**	**0**	**3.2**	**0**			
mean score	**8.4**	**9.4**	**9.8**	**7.2**	**8.0**	**6.7**	**8.6**	**7.5**	**6.9**	**6.7**	**2.5**	**7.2**	**6.0**			
max score	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**7.5**	**10**	**10**			
• **Sample SLS-2.** *Reference Cl- value: 39.53 mEq/L; expected interpretation: "intermediate result which requires further cystic fibrosis assessment"*																
Reporting information	8.0	10.0	9.5	3.5	10.0	10.0	10.0	10.0	10.0	10.0	6.0	8.5	8.0	**3.5**	**8.7**	**10**
Chloride concentration value	0.7	9.3	6.9	7.9	9.2	0.0	0.0	3.7	0.0	9.2	0.0	7.9	0.0	**0**	**4.2**	**9.3**
Clinical sensitivity	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	0.0	10.0	0.0	**0**	**8.5**	**10**
min score	**0.7**	**9.3**	**6.9**	**3.5**	**9.2**	**0**	**0**	**3.7**	**0**	**9.2**	**0**	**7.9**	**0**			
mean score	**6.2**	**9.8**	**8.8**	**7.1**	**9.7**	**6.7**	**6.7**	**7.9**	**6.7**	**9.7**	**2.0**	**8.8**	**2.7**			
max score	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**6**	**10**	**8**			
• **Sample SLS-3.** *Reference Cl- value: 200 mEq/L; expected interpretation: "non-physiological value: results should be questioned"*																
Reporting information	9.5	10.0	9.5	3.5	10.0	10.0	10.0	10.0	10.0	10.0	6.0	8.5	7.0	**3.5**	**8.8**	**10**
Chloride concentration value	1.9	7.4	3.7	8.0	5.8	8.0	0.9	8.2	7.4	6.8	1.2	9.1	9.7	**0.9**	**6.0**	**9.7**
Clinical sensitivity	10.0	10.0	0.0	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	**0**	**3.1**	**10**
min score	**1.9**	**7.4**	**0**	**0**	**0**	**8**	**0**	**0**	**7.4**	**0**	**0**	**0**	**0**			
mean score	**7.1**	**9.1**	**4.4**	**3.8**	**5.3**	**9.3**	**3.6**	**6.1**	**9.1**	**5.6**	**2.4**	**5.9**	**5.6**			
max score	**10**	**10**	**9.5**	**8**	**10**	**10**	**10**	**10**	**10**	**10**	**6**	**9.1**	**9.7**			
TOTAL MAX SCORE FOR ALL CATEGORIES = 100	**71.9**	**94.7**	**78.8**	**61.1**	**78.8**	**77.9**	**66.5**	**74.3**	**78**	**75.9**	**30.6**	**75.6**	**52.6**	**30.6**	**70.5**	**94.7**

In 2016 almost all laboratories except laboratory *p* (score = 0, first time participating) obtained good results and rank was from 6.2/10 to 10/10.

As regards clinical sensitivity, two laboratories (*k* and *m*) obtained a critical score in interpretation in 2015. A poor score was assigned to laboratory *k* due to a not appropriate use of adopted reference intervals while laboratory *m* completely missed the interpretation of results. In 2016, three laboratories (laboratories *k, p, q*) missed interpretation of results and were marked with a "0" score. Laboratories *p* and *q* participated for the first time.

SLS-3

2015: Reference value 200.00 mEq/L; Mean value: 195.95 mEq/L; interpretation: "non-physiological value: results should be questioned"

2016: Reference value 100.00 mEq/L; Mean value: 115.45 mEq/L; interpretation: "supports a diagnosis of cystic fibrosis"

In 2015 chloride concentration results ranked from 0.9/10 to 9.7/10 (mean score = 6.0/10). Only laboratory *f* made a wrong chloride titration (0.9/10).

In 2016 chloride concentration results ranked from 0/10 to 10/10 (mean score = 5.1/10); three laboratories obtained a critical score (namely laboratories *g, k* and *p*).

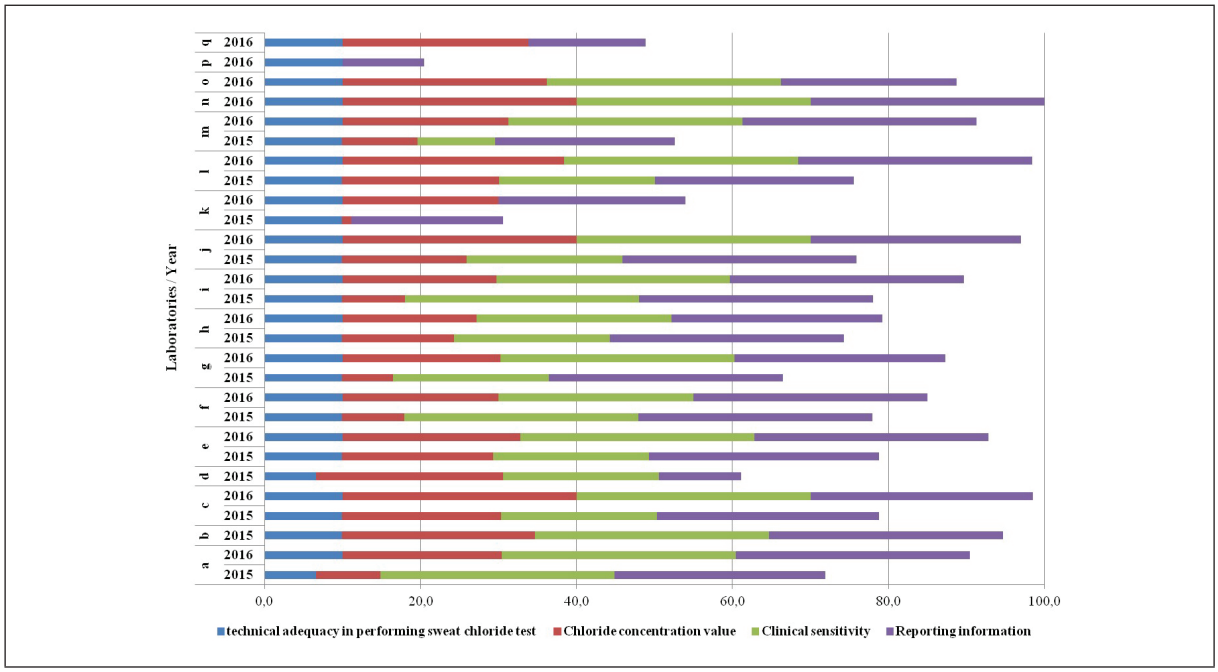

Figure 1
I-EQA-SCT2015/2016 score per parameter.

As regards clinical sensitivity, in 2015, 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. In 2016, three laboratories (*k, p, q*) obtained a 0 score due to the lack of interpretation of results.

Reports assessment

Both in 2015 and 2016 almost all reports were incomplete, missing information concerning one or more parameters. Total scores ranged from 0/30 to 30/30 (median score = 20.8) in 2015 and 10.5/30 to 30/30 (median score = 26.1) in 2016 *(Figure 1)*. In 2015 most frequently missing information concerned "interpretation of results" (15.4% in sample SLS-1; 30.8% in sample SLS-2 and 15.4% in sample SLS-3), "reference intervals" (23.0%, in SLS-1 and SLS-2, 30.8% in SLS-3), and "weight and volume of sweat collected" (15.4% in SLS-1 and SLS-2, 23% in SLS-3).

In 2016 lacking information was referred to "date of primary sample collection" (33.3%, in SLS-1, SLS-2 and SLS-3), "analytical method" (20% in SLS-1, SLS-2 and SLS-3) and "interpretation of results" (20% in all samples).

Poor performance

Three laboratories were marked as poor performers (*k, p* and *q*). Laboratory *k* participated to both 2015 and 2016 EQA-SCT rounds: poor performance was assigned due to lack of interpretation of results in all samples (SLS-1, SLS-2 and SLS-3). Furthermore, this laboratory obtained a 0 score in chloride concentration titration for sample SLS-3. Its final score was 54/100.

Laboratories *p* and *q* participated for the first time in 2016: laboratory *p* was marked as poor performer due to both an insufficient score in chloride concentration titration (score = 0 in SLS-1, SLS-2 and SLS-3) and to the lack of interpretation of results for all the samples; its final score was 20/100.

Laboratories *q* also was marked as a poor performer due to lack of interpretation of results and its final score was 49/100.

DISCUSSION

Diagnostic testing in CF is based on the SCT in the context of appropriate signs and symptoms of disease and results of the gene mutation analysis; so far in fundamental to monitor patients during studies or treatments with CFTR correctors/potentiators/amplifiers, where accurate results are required. Recent papers identify different issues about laboratories standardization in execution, interpretation and reporting of results [6, 7, 11-13].

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 [8] and thereafter the first official round started and was completed.

Ten laboratories participated in the 2014 pilot round; thirteen laboratories were enrolled in 2015 and sixteen in 2016. Seven laboratories participate constantly since 2014 and eleven since 2015. It is not possible to state the exact percentage of laboratories performing SCT and participating in the Italian EQA-SCT, since there is no updated census available; a dedicated working group of the Italian Cystic Fibrosis Society is currently working on it. Therefore, we can only say that about 30% of Italian laboratories, belonging to the public cystic fibrosis Referral Care Centers, are currently monitored for

Table 4
Italian EQA-SCT 2016 general results

	PARTICIPANTS IN 2016 Italian EQA-SCT ROUND																	
	a	***c***	***e***	***f***	***g***	***h***	***i***	***j***	***k***	***l***	***m***	*n*	*o*	*p*	*q*	*min score*	*mean score*	*max score*
Technical adequacy in performing sweat chloride test																		
Stimulation method	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	**3.3**	**3.3**	**3.3**
Sweat collection	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	**3.3**	**3.3**	**3.3**
Analytical method	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	**3.3**	**3.3**	**3.3**
min score	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**			
mean score	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**			
max score	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**			
Quantitative analytical performance																		
Sample SLS-1. *Reference Cl^- value: 35.66 mEq/L; expected interpretation: "intermediate result which requires further cystic fibrosis assessment"*																		
Reporting information	10.0	9.5	10.0	10.0	9.0	9.0	10.0	9.0	8.0	10.0	10.0	10.0	7.5	2.5	5.0	**2.5**	**8.6**	**10.0**
Chloride concentration value	8.3	10.0	6.2	0.0	10.0	10.0	6.7	10.0	10.0	10.0	10.0	10.0	10.0	0.0	7.6	**0.0**	**7.9**	**10.0**
Clinical sensitivity	10.0	10.0	10.0	5.0	10.0	10.0	10.0	10.0	0.0	10.0	10.0	10.0	10.0	0.0	0.0	**0.0**	**7.7**	**10.0**
min score	**8.3**	**9.5**	**6.2**	**5.0**	**9.0**	**9.0**	**6.7**	**9.0**	**0.0**	**10.0**	**10.0**	**10.0**	**7.5**	**0.0**	**0.0**			
mean score	**9.4**	**9.8**	**8.7**	**5.0**	**9.7**	**9.7**	**8.9**	**9.7**	**6.0**	**10.0**	**10.0**	**10.0**	**9.2**	**0.8**	**4.2**			
max score	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**8.0**	**10.0**	**10.0**	**10.0**	**10.0**	**2.5**	**7.6**			
Sample SLS-2. *Reference Cl^- value: 54.96 mEq/L; expected interpretation: "intermediate result which requires further cystic fibrosis assessment"*																		
Reporting information	10.0	9.5	10.0	10.0	9.0	9.0	10.0	9.0	8.0	10.0	10.0	10.0	7.5	4.0	5.0	**4.0**	**8.7**	**10.0**
Chloride concentration value	9.1	10.0	10.0	10.0	10.0	6.2	10.0	10.0	10.0	10.0	10.0	10.0	10.0	0.0	10.0	**0.0**	**9.0**	**10.0**
Clinical sensitivity	10.0	10.0	10.0	10.0	10.0	5.0	10.0	10.0	0.0	10.0	10.0	10.0	10.0	0.0	0.0	**0.0**	**7.7**	**10.0**
min score	**9.1**	**9.5**	**10.0**	**9.0**	**9.0**	**5.0**	**10.0**	**9.0**	**0.0**	**10.0**	**10.0**	**10.0**	**7.5**	**0.0**	**0.0**			
mean score	**9.7**	**9.8**	**10.0**	**10.0**	**9.7**	**6.7**	**10.0**	**9.7**	**6.0**	**10.0**	**10.0**	**10.0**	**9.2**	**1.3**	**5.0**			
max score	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**9.2**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**4.0**	**10.0**			
Sample SLS-3. *Reference Cl^- value: 100.00 mEq/L; expected interpretation: "supports a diagnosis of cystic fibrosis"*																		
Reporting information	10.0	9.5	10.0	10.0	9.0	9.0	10.0	9.0	8.0	10.0	10.0	10.0	7.5	4.0	5.0	**4.0**	**8.7**	**10.0**
Chloride concentration value	3.0	10.0	6.6	10.0	0.3	1.0	3.0	10.0	0.0	8.4	1.3	10.0	6.2	0.0	6.2	**0.0**	**5.1**	**10.0**
Clinical sensitivity	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	0.0	10.0	10.0	10.0	10.0	0.0	0.0	**0.0**	**8.0**	**10.0**
min score	**3.0**	**9.5**	**6.6**	**10.0**	**0.3**	**1.0**	**3.0**	**9.0**	**0.0**	**8.4**	**1.3**	**10.0**	**6.2**	**0.0**	**0.0**			
mean score	**7.7**	**9.8**	**8.9**	**10.0**	**6.4**	**6.7**	**7.7**	**9.7**	**2.7**	**9.5**	**7.1**	**10.0**	**7.9**	**1.3**	**3.7**			
max score	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**10.0**	**8.0**	**10.0**	**10.0**	**10.0**	**10.0**	**4.0**	**6.2**			
TOTAL MAX SCORE FOR ALL CATEGORIES = 100	**90.0**	**98.0**	**93.0**	**85.0**	**87.0**	**79.0**	**90.0**	**97.0**	**54.0**	**98.0**	**91.0**	**100.0**	**89.0**	**20.0**	**49.0**	**20.0**	**81.3**	**100.0**

Laboratories in bold participated to both 2015 and 2016 I EQA-SCT round. Laboratories highlighted in grey (k. p and q) were assessed with a "poor performance" score

CF SCT. In order to increase the number of volunteers participants in the 2017 round, the program was promoted through ISS and scientific societies websites as well as discussed results in dedicated CF meeting sessions at national level.

Quantitative analytical performance in both 2015 and 2016 was characterized by a significant heterogeneity.

In particular, there was a variability in scores as regards the evaluation of chloride titration both in 2015 and in 2016; 16 errors in Cl^- titration (score = 0) were made by eight different participating laboratories. Three laboratories (*f*, *g* and *k*) participated to both 2015 and

2016 schemes. 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.

Four laboratories (*a, j, i,* and *m*) made a wrong chloride titration in 2015, but drastically improved their performance in 2016 by 45% on average. Laboratory *p* made an error and participated for the first time in 2016.

Currently we cannot exclude that errors are due to methodological, equipment or technical problems caused by the unskilled personnel [14] but data referred to the four laboratories that improved their performance encourage a *long-lasting* participation.

In 2016 poor performance criteria were established and adopted and three laboratories (*k*, *p* and *q*) received such a categorization.

Poor-performing laboratories were encouraged to review their internal processes and to contact a dedicated working group within the Italian Cystic Fibrosis Society. Single occurrences of poor performance should be logged as an incident and used as an opportunity to review procedures and make improvements. A careful evaluation of the error may determine whether there is a system failure that may require re-design of a test, more frequent instrument calibration, or adjustments to training procedures. However, EQA may detect serial or persistent failures that the laboratory is obligated to address with a more fundamental review [15] and it will be interesting to monitor eventual failure in future rounds.

As regards clinical sensitivity, in 2015 about 70% of laboratories failed to make suggestions when the chloride value was reported as not physiological (sample SLS-3) as clearly indicated by National Guidelines [5]. In 2016 three laboratories (*k*, *p* and *q*) obtained a 0 score in clinical sensitivity assessment due to the complete lack of interpretation of results in all samples sent by the provider. Noteworthy, in two out of three cases (laboratory *k* and *q*) analytical results were correct *(Table 4)*.

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 EQA participation should become mandatory as a component of laboratory accreditation, the quality of laboratory performance is unpredictable [16].

All data collected highlight the need and the importance to continue this activity constantly in order to support laboratories performing sweat chloride test and to ensure adequate quality standards.

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Conflict of interest statement

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Ethical approval (including reference number)

Ethical approval was not required because all data are kept anonymous and clinical information about samples is mock.

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Italian EQA-SCT Working Group

Ettore Capoluongo Laboratorio di Diagnosi Clinico Molecolare e Personalizzata, Fondazione Policlinico Universitario "A. Gemelli", Rome, Italy; **Ubaldo Caruso**, LABSIEM – Laboratorio per lo Studio degli Errori Congeniti del Metabolismo, Università degli Studi di Genova – DINOGMI, Genoa, Italy; **Giuseppe Castaldo**, CEINGE – Biotecnologie Avanzate Scarl, Napoli, Italia and Dipartimento di Medicina Molecolare e Biotecnologia Medica, Università degli studi di Napoli Federico II, Naples, Italy; **Natalia Cirilli**, Centro di Riferimento Fibrosi Cistica, Dipartimento Materno Infantile, Ospedali Riuniti Salesi – Ospedale Pediatrico, Ancona, Italy; **Carlo Corbetta**, SC Laboratorio di Riferimento Regionale per lo Screening Neonatale – Regione Lombardia, ASST Fatebenefratelli Sacco, Milan, Italy; **Rita Padoan**, Centro di Supporto Fibrosi Cistica, Dipartimento Pediatrico, Ospedale Pediatrico, ASST Spedali Civili, Brescia, Italy; **Valeria Raia**, Centro Regionale Fibrosi Cistica, Unità di Pediatria, Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli, Federico II, Naples, Italy.

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