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Implementation of the First Worldwide Quality Assurance Program for Cystic Fibrosis Multiple Mutation Detection in Population-Based Screening

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Abstract

Background—CDC's Newborn Screening Quality Assurance Program collaborated with several U.S. Cystic Fibrosis Care Centers to collect specimens for development of a molecular *CFTR* proficiency testing program using dried-blood spots for newborn screening laboratories.

Methods—Adult and adolescent patients or carriers donated whole blood that was aliquoted onto filter paper cards. Five blind-coded specimens were sent to participating newborn screening laboratories quarterly. Proficiency testing results were evaluated based on presumptive clinical assessment. Individual evaluations and summary reports were sent to each participating laboratory and technical consultations were offered if incorrect assessments were reported.

Results—The current CDC repository contains specimens with 39 different *CFTR* mutations. Up to 45 laboratories have participated in the program. Three years of data showed that correct assessments were reported 97.7% of the time overall when both mutations could be determined. Incorrect assessments that could have lead to a missed case occurred 0.9% of the time, and no information was reported 1.1% of the time due to sample failure.

Conclusions—Results show that laboratories using molecular assays to detect *CFTR* mutations are performing satisfactorily. The programmatic results presented demonstrate the importance and complexity of providing proficiency testing for DNA-based assays.

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Keywords

Newborn Screening; Proficiency Testing; Cystic Fibrosis; DNA; mutations

INTRODUCTION

The U.S. Centers for Disease Control and Prevention (CDC) Newborn Screening Quality Assurance Program (NSQAP) is a comprehensive program serving newborn screening (NBS) programs in the U.S. and around the world. For over 31 years NSQAP has provided quality control (QC) and proficiency testing (PT) materials in the dried-blood spot (DBS) matrix as well as training, consultations, and filter paper evaluations. As of December 31, 2009, NSQAP provided QC and/or PT materials for 33 analytes covering all primary and most secondary targets on the U.S. Secretary's Advisory Committee for Heritable Disorders in Newborns and Children recommended uniform screening panel[1]. These materials were sent to 440 laboratories in 61 countries, including all U.S. NBS laboratories[2]. Most NBS assays utilize biochemical methods to detect a variety of genetic disorders; however cystic fibrosis (CF) screening often incorporates a second-tier molecular test that identifies region appropriate CF-causing mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Thus, it was essential that NSQAP develop new strategies to provide a proficiency testing program for NBS laboratories that can accommodate the variable CF multi-mutation assays.

CF is an autosomal recessive, life-threatening disease with an average incidence of approximately 1:4,000 in Western Europe, North America, and Australasia. NBS to detect CF first became feasible after 1979 when researchers in New Zealand discovered that DBS specimens from newborns that were later diagnosed with CF by a sweat test were shown to have high levels of immunoreactive trypsinogen (IRT)[3]. Soon thereafter, countries such as Australia and the U.S. state of Colorado implemented IRT/IRT testing, which involved an initial IRT measurement followed by analysis of a second specimen at approximately 2 weeks of age to determine if the hypertrypsinogenemia was persistent enough for diagnostic referral [4,5].

However, because of concerns about suboptimal sensitivity of the initial IRT cutoffs and observations revealing that repeat (recall) specimens of some CF patients showed precipitous decreases in IRT, Wisconsin developed the two tier IRT/DNA method. This method used a lower IRT cutoff followed by detection of the p.Phe508del mutation in the *CFTR* gene [6]. Wisconsin's IRT/DNA screening results gave better sensitivity than their IRT/IRT program and clinical data indicated significant benefits from early diagnosis, resulting in Wisconsin's implementation of routine IRT/DNA screening in 1994[7]. In 1999, the New England Newborn Screening Program began using a multiple mutation panel for *CFTR* mutations in order to increase their sensitivity further, capturing other mutations seen in the Massachusetts population [8]. This mutation detection expansion increased the test's sensitivity, as well as the number of *CFTR* mutation carriers detected.

In October 2004, CDC published a pivotal recommendation that U.S. states begin CF NBS based on results from a randomized controlled trial which showed better nutritional and

growth outcomes among screened children, as well as risk-benefit analyses that evaluated experienced CF NBS programs [9]. With the inclusion of CF on the U.S. recommended uniform newborn screening panel in 2006, states began to implement CF screening. By December 2009, all states and the District of Columbia were screening for CF using an IRT/IRT algorithm (N=16) or an IRT/DNA algorithm (N=35).

As more U.S. states began CF NBS, quality assurance became increasingly important. NSQAP began offering IRT PT materials in 2002 and QC materials in 2006. With the increase in the number of laboratories using multi-mutation *CFTR* assays in CF NBS, NSQAP recognized the need for a *CFTR* mutation PT program. This program presented unique challenges for several reasons: (1) there are a multitude of different mutation panels being used in the DNA component of the IRT/DNA algorithm; (2) there are many commercially available and in-house methods being used to detect these different *CFTR* mutation panels; and (3) there is limited availability of suitable challenge specimens. Hence, NSQAP, in collaboration with U.S. CF care centers, implemented a PT program that provides NBS laboratories with blind-coded DBS specimens prepared from volunteer donors that cover the most common *CFTR* mutations including but not limited to the American College of Medical Genetics (ACMG) recommended 23 mutations [10,11]. This study describes the design, implementation, and encouraging outcomes of this PT program for DNA-based methodologies used in NBS for CF detection. This is the first application of a worldwide multiple mutation DNA PT program for population-based screening.

MATERIALS AND METHODS

Overall Design of the Pilot PT Program

The overall development of this program is described in Figure 1.

Specimen Collection

Whole blood was collected voluntarily from adult or adolescent CF patients and/or parents into 10 mL EDTA tubes (Becton Dickinson, Thomas Scientific, Swedesboro, NJ) and mixed well by inversion. Tubes were labeled with a specimen number and genotype, and then shipped overnight to CDC with cold packs. Upon arrival, the hematocrit of the blood was adjusted to 50-55% and 75 μ L aliquots of blood were spotted onto Whatman 903 filter paper cards (Piscataway, NJ). Cards were dried overnight at room temperature and stored at -20°C with desiccant and humidity indicator cards.[12] The non-CF DBS specimens were made from a unit of whole blood collected in EDTA tubes from a donor who had been found to be negative for 31 *CFTR* mutations. Three CF care centers located in Baltimore MD, Cleveland OH, and Madison WI initially participated in establishing this program, and others have contributed blood specimens subsequently. The project was determined to be research exempt by the U.S. CF Care Centers Institutional Review Board. CDC's Human Research Protection Office does not consider the repository to be human research as all specimens are de-identified and cannot be traced back to the donor by CDC or NBS laboratories.

Confirmation of Genotype from DBS PT Materials

All genotype confirmations or identifications were performed by the Newborn Screening and Molecular Biology Branch which includes NSQAP and the Molecular Risk Assessment Laboratory. DNA was extracted from a 3.2 mm DBS punch using Qiagen's Generation DNA Purification and Elution Solutions (formerly manufactured by Gentra Corp.) according to the manufacturer's protocol (Valencia, CA). In some cases when a higher DNA concentration was needed, DNA was extracted from a 250 µl aliquot of whole blood using Qiagen's QIAmp DNA Blood mini-kit according to manufacturer's instructions.

The *CFTR* genotypes were confirmed using Innogenetics *CFTR*36 Probe assay (Innogenetics, Gent, Belgium). Two multiplexed amplification reactions, each containing 5 µL template DNA, 10 µl Amplification Buffer, 10 µl Primer Set 1 or 2, 0.85 µl Taq Hot Star Polymerase (Qiagen) and 24.15 µl nuclease-free water (Invitrogen, Carlsbad, CA) were prepared. The amplification involved an initial cycle of 94°C for 15 min followed by 30 cycles of 94°C for 1 min, 57°C for 1 min, and 68°C for 30 sec. After a final cycle of 68°C for 10 min, the reaction was stored at 4°C until used processing. Amplified products were electrophoresed on a 10% acrylamide gel to verify amplification. Hybridization of the amplified products to the *CFTR* probes was performed on the MedTec 3000 instrument using the *CFTR* program installed by the vendor (Innogenetics, Gent, Belgium).

The *CFTR* genotypes for some specimens were confirmed using Asuragen Signature® *CFTR* Mutations RUO Assay (Asuragen, Austin, TX). Each amplification reaction contained 5 µl template DNA, 5 µl amplification buffer, 5 µl primers, 1 µl AmpliTaq Gold (5 U/µl) (Applied Biosystems, Foster City, CA), and 34 µl Diluent. Amplification parameters were 1 cycle at 37°C for 15 min then and 95°C for 10 min followed by 35 cycles of 94°C for 30 sec, 58°C for 90 sec, and 72°C for 90 sec. After a final cycle of 72°C for 2 min, the reaction was stored at 4°C. The amplified products were used within 72 h from the completion of the thermal cycling. Each 50 µl hybridization reaction contained 48 µl of the *CFTR* probes and 2 µl of amplified product and was allowed to hybridize for 1 cycle at 95°C for 5 min, 1 cycle at 52°C for 25 min, and 1 cycle at 52°C for up to 10 minutes. The hybridization plate was quickly transferred to the Luminex® 200 (Luminex, Austin, TX) metallic plate which was pre-warmed to 52°C. The conjugate was diluted 1:50 in the buffer provided by Asuragen and 25 µL was added to each reaction well. Data was collected by the Luminex® instrument and allele calls were generated by the Asuragen® Signature Script® *CFTR* Software.

Specimens with only 1 known mutation were further characterized by sequencing the promoter, exons, intron/exon boundaries and the 3' end of the gene using Applied Biosystems VariantSeq™ Resequencing System and additional custom designed primer pairs or previously published primer pairs for complete coverage of the *CFTR* gene [13,14]. Deletions not included in the Innogenetics *CFTR* Probe assay were identified or confirmed with the MRC-Holland Multiplex Ligation-dependent Probe Amplification (MLPA) kit (Amsterdam, Netherlands) according to manufacturer's instructions.

Distribution of PT Specimens

Participation was open and free of charge to all NBS laboratories worldwide that use mutation detection as part of their CF screening algorithm. Current participants of NSQAP's IRT PT Program were invited to participate in the *CFTR* mutation detection PT program. New participants were added when they started CF NBS in their laboratories or upon request. Five blind-coded DBS specimens were sent quarterly and participants were given 4 weeks from the date of shipment to report results via email or fax.

PT Instructions to Participants

Participants were instructed to treat the specimens as if they were from full-term newborns with an IRT measurement above the laboratory's cutoff. Laboratory staff processed and tested the specimens as they would newborn specimens suspected of having CF. Data reported back to NSQAP included the method used, the genotype found, and a presumptive clinical assessment for each specimen. Presumptive clinical assessments included the following: screen negative (normal); 2 mutations detected, likely CF; or 1 mutation detected, likely CF carrier.

Evaluation

Participants were evaluated based on the reported presumptive clinical assessment. Due to the variety of methods, algorithms, and mutations assessed worldwide, a specimen was considered not evaluated by NSQAP when the reported method was known not to detect one or both mutations in a challenge specimen. Sample failure describes any event during testing where a result could not be reported (amplification failure, assay failure, etc.).

Summary Reports

Participants received a summary report and individual evaluations via mail. The summary report contained the expected results and a compilation of the participants' results, including the frequency of presumptive clinical assessments, the number of errors, and the number of results that were considered not evaluated. If laboratory staff reported an incorrect presumptive clinical assessment, they were alerted by telephone (US participants) or email (international participants) and offered assistance or consultation.

RESULTS

Yield of Blood Collection Activities

The NSQAP Repository contains 39 unique *CFTR* mutations including all of the ACMG 23 recommended mutations for CF population screening. Most specimens are compound heterozygotes (Table 1) and all provided sufficient quantities of DBS for this program. Seven specimens were received in 2006, 51 in 2007, 10 in 2008, and 8 in 2009. Specimens may have been collected from the same donor more than once depending on their desire to donate. Only one specimen had an incorrect genotype reported by the U.S. CF Care Center. Further investigation by the U.S. CF Care Center determined that the genotype was correct in the patient's file, but had been incorrectly reported to NSQAP. In five specimens, the second mutation had been unknown most likely because *CFTR* had been genotyped many

years ago when many of the mutations were still not recognized. In these instances, CDC was able to determine the unknown mutation. Specimen collection is ongoing and NSQAP is continuing partnerships with U.S. CF Care Centers to sustain the repository and the PT program into the future.

Participating Laboratories

As of December 31, 2009, forty-four laboratories worldwide participated in NSQAP's *CFTR* mutation detection PT program, including 26 programs in the U.S. and Canada, 12 in Europe, 2 in South America, and 4 in Australia and New Zealand. Up to 18 different methods, including different commercial kits, analyte-specific reagents from a variety of manufacturers and in-house assays were reported. Instrumentation, such as real-time PCR instruments, Luminex X-map, matrix assisted laser desorption/ionization – time of flight mass spectrometer (MALDI-TOF), Roche Lightcycler, and plate spectrophotometers were used for detection. Variable numbers of mutations were detected by the participating laboratories that utilize a variety of different platforms (Table 2).

CF NBS Algorithms

Different screening algorithms and mutation panels are used by NBS laboratories both nationally and internationally. The majority of NBS programs follow a screening algorithm that tests the specimens for multiple mutations contained in their specified panel after an elevated (above their cutoff) IRT measurement. If one or more mutation(s) is found, the baby is considered a screen positive. Some programs limit their mutation detection to the more common p.Phe508del mutation in Exon 10 of the *CFTR* gene after an elevated IRT measurement. If no p.Phe508del mutation is found, no further testing is done. If one p.Phe508del mutation is found, further genetic analysis is completed. A third algorithm tests specimens for four common mutations after an elevated IRT measurement. Again, if no mutations are found, no further testing is done. If one mutation is found, the specimen is tested for a more comprehensive mutation panel. Each laboratory provided NSQAP with the algorithm they use and the panel of mutations they analyze so that their presumptive clinical assessments could be evaluated against their specific protocol.

Outcomes of PT

NSQAP considers a specimen gradable when at least 80% of U.S. participants can report the correct assessment. All specimens were gradable throughout this time period. Correct assessments were reported >90% of the time by laboratories that employed methods that could detect both mutations for all specimens (Table 3). The average number of correct assessments for the 12 quarterly PT assessments, each containing 5 challenge specimens, was 97.7%. Incorrect assessments occurred <10% of the time for any given quarter and incorrect assessments that could have lead to a missed case occurred only 0.6% of the time. When a participant reported an incorrect assessment, NSQAP contacted the laboratory to identify the source of the error (Table 4). Almost half of the incorrect assessments (41%) were the result of transcription errors when filling out the NSQAP data report form. For three of the incorrect assessments (8%), the NBS program modified the standard panel of mutations detected by the chosen method but did not inform NSQAP.

The total number of specimens tested from 2007-2009, the percentage of specimens not evaluated because one or both of the alleles were not included in a participant's mutation assay and the percentage not reported due to a sample failure is summarized in Table 5. Overall, 15.4% of the specimens were not evaluated demonstrating the variety of mutation panels used by participants. Only 1.1% of the results were classified as sample failures, which are defined as any event that would not allow a presumptive clinical assessment.

DISCUSSION

Quality performance in the laboratory is critical to produce reliable results. Periodic audits like NSQAP's PT programs promote continuous quality improvement and are especially important for laboratory staff implementing new techniques, such as molecular testing. Before routine CF molecular NBS, relatively few NBS laboratories had the instrumentation and expertise to conduct molecular screening. The CF Mutation Detection PT Program provides an independent external assessment of the laboratory's performance while providing summary information about available methods. In addition to embracing molecular technology, when NBS laboratories added CF to their screened disorders, many had to closely interact with a new partner, the CF care centers. To ensure the performance and reliability of the NBS results, the NBS laboratories needed a PT program designed to assure quality molecular results with the DBS matrix. Therefore, the PT program had to assess all aspects of testing, from DNA extraction through mutation detection. NBS laboratories participation in NSQAP's CF Mutation Detection PT Program has demonstrated satisfactory performance in detecting *CFTR* mutations which has been communicated to collaborating CF care centers and other specialists.

Providing PT for DNA-based tests is a complicated venture since there are many assays that detect different mutation panels as well as an assortment of testing methods and testing algorithms. Conversely, the traditional biochemical and immunological assays used in NBS do not have the same issues found with DNA-based mutation detection assays. For instance, NSQAP's acylcarnitine PT program had 200 participants at the end of 2009, but only 6 methods, all of which use tandem mass spectrometry. Similarly, NSQAP's IRT PT program had 132 participants at the end of 2009 with only 5-8 methods, all of which were enzyme immunoassays. The *CFTR* mutation detection PT program has had as many as 18 different assays from 45 laboratories, a subset of which use multiple assays within a single laboratory. The number of assays and the variety of mutation panels can significantly increase with new participants. Since participants are currently analyzing any number of mutations ranging from one mutation (p.Phe508del) to hundreds, the PT program must accommodate most of the panels and algorithms in order to be effective. Interestingly, the common *CFTR* mutation, p.Phe508del, is the only mutation common to all 45 laboratories' panels. Thus, NSQAP's CF Mutation Detection PT Program was designed to cover a wide variety of mutations, and to ensure that laboratories can detect mutations that are not routinely seen, regardless of the algorithm or methods used.

Neonatal DBS are a limited resource and in order to sustain the PT program, patient blood must continue to be collected. NSQAP is committed to developing and sustaining partnerships with U.S. CF Care Centers to increase the yield and diversity of the specimens.

While it is impractical for NSQAP to collect blood from very rare or private mutations which are unlikely to be seen in population screening, it is feasible to cover the U.S. panel of 23 mutations and a reasonable number of other important mutations to appropriately serve NBS laboratories.

The 3 years of PT results presented show that NBS laboratories using mutation detection methods for second-tier CF testing are performing well. Since NBS laboratories must accept a higher false positive rate to avoid missing a true positive, patients are referred for further testing when only one mutation is found. Analysis of the incorrect assessments over the three year time period found that only 14 (0.9%) of the 37 incorrect assessments would have led to a missed case. In addition, only 4 of these 14 incorrect assessments were a true miss in which no mutation was detected in a specimen that was from a CF carrier or patient. Thus, more than 91% of laboratories did not make an incorrect assessment that would have led to a missed CF case. These mistakes occurred in individual laboratories and did not have any common genotypes. The remaining 10 errors were due to transcription errors. In these cases, the correct mutations were detected but the wrong code was reported on the NSQAP data report form. The faxed/emailed report form associated with this PT program does not accurately measure the likelihood of actual transcription errors since NBS laboratory reporting typically uses an automated system. Hence, the transcription error rate seen in this program is potentially artificially elevated, reinforcing the importance of an automated report.

As described in Table 4, there are a variety of reasons for incorrect assessments. The three incorrect assessments that should have been classified as “not evaluated” because the laboratory does not report those mutations, illustrates the importance of communication between participants and providers. This is especially true for multi-mutation PT programs where the use of different panels is common. Some of the incorrect assessments were attributable to DNA extraction issues leading to an inadequate amount of DNA or compromised quality, making it insufficient or invalid for analysis. Previous results from a study using a subset of specimens from the NSQAP *CFTR* mutation detection repository showed that the DNA concentration from a 3.2 mm punch eluted in a volume of 50 µl ranged from 1.6-7.0 ng/µl as assessed by the Applied Biosystems Quantifiler® Duo forensic assay.[15] While there is a broad range of acceptable DNA concentrations that work with most commercially available CF mutation detection assays, if the DNA extraction is not optimal then amplification failure, assay failure, or incorrect genotyping could result, especially for the more complicated multiplex assays. Those laboratories that reported a method issue, including low DNA quality would, would result in an incorrect presumptive result, which may lead to a child being missed.

The error rates seen in NSQAP's *CFTR* mutation detection PT program are comparable to other PT programs that are more specifically designed for diagnostic laboratories testing for *CFTR* mutations to confirm a CF diagnosis. The Italian External Quality Control Programme for CF, which operated from 2001 through 2004, sent 6 specimens of purified DNA to participating laboratories once a year for *CFTR* mutation detection. The 24 validated DNA specimens included 21 heterozygous or compound heterozygous specimens and 3 carrier specimens. The genotyping error rate was approximately 0.7% over 4 y with

approximately half due to interpretive errors and 45% due to analytical errors [16]. The College of American Pathologists (CAP) also has a *CFTR* mutation detection PT program that provides 2-5 specimens of purified DNA/y. Their reported results confirm a high rate of correct genotyping, however it will not assess any errors due to DNA extraction or the resulting purified DNA from the DBS matrix. Thus, in order to provide a comprehensive quality assessment of NBS laboratories engaged in *CFTR* mutation detection, NSQAP's offers 20 challenge specimens per year in the DBS matrix. Three years of data show a similarly low reported error rate to other *CFTR* mutation detection PT programs, demonstrating that NBS laboratories are able to reliably extract DNA from very small quantities of blood on filter paper and provide quality results similar to diagnostic laboratories regardless of the genotyping method used.

A secondary objective for this program was to establish and maintain a repository of CF patient specimens with a wide variety of genotypes for method development and validation in NBS laboratories. Since many of the mutation panels in use have low population frequencies, it can be difficult to obtain appropriate patient specimens for these purposes. Currently, NSQAP's CF Mutation Detection repository contains 39 mutations, including all 23 ACMG recommended mutations. The program continues to work to expand the CF care centers to provide well-characterized specimens with an even greater number of mutations for testing.

The CF Mutation Detection PT Program is an example of a mutually beneficial and successful collaboration between CDC and the Cystic Fibrosis Foundation (CFF) that has lasted for more than ten years. This collaboration began with CDC and CFF discussing the usefulness and readiness for CF NBS and has culminated in a program that provides PT services for CF screening to NBS laboratories worldwide.[17] As molecular testing for NBS disorders increases, this program can serve as a model for future PT programs using DNA-based screening methods in the DBS matrix.

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ABBREVIATIONS

ACMG	American College of Medical Genetics
CFF	Cystic Fibrosis Foundation
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator gene
DBS	dried-blood spot
IRT	Immunoreactive trypsinogen
NBS	Newborn Screening
NSQAP	Newborn Screening Quality Assurance Program

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Fig. 1.

Flow chart of development for proficiency testing program

Table 1

CFTR mutations in the NSQAP repository and U.S. population frequencies (%) [11]

Allele	Allele	Allele	Allele
p.Gly85Glu	p.Arg117His	c.489+1G>T	p.Phe508del
G85E (0.26)	R117H (0.54)	621+1G>T (1.3)	F508del (66.31)
p.Arg347Pro	p.Ile507del	p.Gly551Asp	c.2052delA
R347P (0.36)	I507del (0.90)	G551D (1.93)	2184delA (0.15)
c.1585-1G>A	p.Gly542X	c.3528delC	p.Asn1303Lys
1717-1G>A (0.44)	G542X (2.64)	3659delC (0.28)	N1303K (1.27)
p.Arg553X	p.Arg560Thr	p.Arg1162X	c.2657+5G>A
R553X (1.21)	R560T (0.30)	R1162X (0.30)	2789+5G>A (0.38)
c.3717+12191C>T	c.2988+1G>A	p.Trp1282X	p.Ala455Glu
3849 + 10kbC>T (0.85)	3120+1G>A (0.86)	W1282X (2.20)	A455E (0.26)
c.1766+1G>A	c.579+1G>T	p.Arg334Trp	c.54-5940_273+10250del21kb
1898+1G>A (0.13)	711+1G>T (0.35)	R334W (0.37)	CFTR dele2,3
p.Ser549Asn	c.1584G>A	c.2051 2052delAAinsG	c.3140-26A>G
S549N (0.14)	1716G→A	2183AA>G (0.1)	3272-26A>G
c.262_263delTT	p.Arg1066Cys	p.Arg1066His	c.1022_1023insTC
394delTT	R1066C (0.03)	R1066H	1154insTC
c.2989-1G>A	c.(?_2989)_(3139_?)del	p.Leu467Pro	p.Gln39X
3121-1G>A	Exon 17a-17b del	L467P	Q39X
c.(?_54)_(164_?)del	c.3773_3774insT	c.(?_2989)_(3367_?)del	
Exon 2del	3905insT (0.12)	Exon 17a-17b-18 del	

Mutation legacy names and frequency (%) in the U.S. population (if known) are listed below the standard name.

Table 2

List of reported methods, number of mutations detected and number of laboratories using each method

Methodology	Number of Mutations Detected	Number of Laboratories
Hologic Inplex® CF Molecular Test	23 or 39	13
Luminex Diagnostics X-Tag®CF Assays	39	8
Gen-Probe Elucigene ARMS Assay	23, 29, or 30	4
Innogenetics <i>CFTR</i> 36 Probe Assay	36	1
Asuragen Signature CF 2.0	45 or 54	2
Abbott Diagnostics	31 (ver.2)	1
Matrix-assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry	Unlimited (theoretical)	1
Sequencing of 5 Exons j^S	>300	1
In-house Heteroduplex Assay for Exon 10 ^a	2	1
In-house Lightcycler® Assay ^a	1	1
In-house TaqMan® Allelic Discrimination Assay	8	1
In-house Single Nucleotide Polymorphism Assay	19	1
In-house hydrolysis probe assay	4	1
Amplification/Heteroduplex/Restriction analysis	9	1
Home-brew Assay	3	1
Amplification / gel electrophoresis ^a	1	3
Roche Diagnostics Linear Array ^b	25	1
Nanogen Cystic Fibrosis Detection kit ^b	23	1

^aUsed in addition to another listed method^bDiscontinued

Table 3

Proficiency testing results summary

Quarter	Specimens Assayed(N) ^a	Correct Assessments N (%)	Incorrect assessments potentially leading to a missed case (%) ^b	
			Transcription Error ^c N (%)	True Miss N (%)
2007				
1	77	76 (99.7)	0 (0.0)	0 (0.0)
2	75	75 (100)	0 (0.0)	0 (0.0)
3	94	91 (96.8)	0 (0.0)	0 (0.0)
4	103	94 (91.3)	1 (1)	0 (0.0)
2008				
1	107	104 (97.2)	2 (2.0)	0 (0.0)
2	124	120 (96.8)	0 (0.0)	1 (0.8)
3	142	138 (97.2)	1 (0.7)	0 (0.0)
4	182	175 (96.2)	6 (3.3)	0 (0.0)
2009				
1	181	179 (98.9)	0 (0.0)	1 (0.6)
2	175	174 (99.4)	0 (0.0)	0 (0.0)
3	161	160 (99.4)	0 (0.0)	1 (0.6)
4	204	202 (99.0)	0 (0.0)	1 (0.5)
Total	1625	1588 (97.7)	10 (0.6)	4 (0.2)

^a If one or both mutations are not part of the laboratory's panel or the specimen did not amplify, then the specimen is not evaluated and is removed from the analysis (See Table 5).

^b For a detailed account of all incorrect results, please refer to Table 4

^c One or two mutations were identified in the specimen but the "screen negative" code was reported on CDC Data Report Form.

Table 4

Reasons for incorrect assessments and incidence of each

Issue	Total (%)	Domestic (%)	International (%)
Transcription Error ^a	15 (41%)	9 (56%)	6 (29%)
No Response ^b	9 (24%)	3 (19%)	6 (29%)
Method issue ^c	4 (11%)	1 (6%)	3 (14%)
Software issue (misinterpretation of calls; +/- only)	3 (8%)	2 (13%)	1 (5%)
Does not report mutation ^d	3 (8%)	1 (6%)	2 (10%)
Low DNA quality	1 (3%)	0 (0%)	1 (5%)
Kit only detects presence not # of copies ^e	1 (3%)	0 (0%)	1 (5%)
Lab manager received results over phone (misinterpreted)	1 (3%)	0 (0%)	1 (5%)
Total	37 (100%)	16 (100%)	21 (100%)

^a Correct genotype but incorrect clinical assessment reported^b Laboratory did not respond to request to describe problem encountered^c True analytical error^d Method can detect mutation but the program asked manufacturer to blind the data because it does not report the mutation as part of the screening protocol (failure to communicate with NSQAP).^e These programs refer all patients with 1 mutation for a sweat test; a child would not be missed

Table 5

Summary of non-evaluated results and sample failure

Quarter	Specimens Assayed (N) ^a	Not Evaluated ^b N (%)	Sample Failure ^c N (%)
2007			
1	110	31 (28.2)	2 (1.8)
2	110	35 (31.8)	0 (0.0)
3	125	26 (20.8)	5 (4.0)
4	140	36 (25.7)	1 (0.7)
2008			
1	140	32 (22.1)	1 (0.7)
2	145	21 (14.5)	0 (0.0)
3	165	23 (13.9)	0 (0.0)
4	195	9 (4.6)	4 (2.1)
2009			
1	205	23 (11.2)	1 (0.5)
2	195	18 (9.2)	2 (1.0)
3	200	34 (17.0)	5 (2.5)
4	215	11 (5.1)	0 (0.0)
Total	1945	299 (15.4)	21 (1.1)

^aTotal number of specimens assayed regardless of evaluation^bIf 1 or more mutations in a challenge specimen is not part of the laboratory's testing panel or a sample failure was reported, the result is not evaluated and is removed from the analysis^cSample failure describes any event where a result could not be reported (amplification failure, assay failure, etc.)