

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

Cystic Fibrosis Mutation Detection
Quarterly Report

Volume 9, No. 4

November 2015

INTRODUCTION

This report is the quarterly summary of all data reported within the specified data-reporting period for the Quarter 4, 2015 program for cystic fibrosis (CF) mutation detection for the Newborn Screening Quality Assurance Program. The attached tables provide the certification profiles for the distributed specimens, the summary of frequencies of clinical assessments reported, the summary of frequencies of reported genotypes, the primary and secondary methods used by participants, the DNA extraction methods used by participants and the verification of your reported data. Methods varied widely with regard to the panel of mutations detected, the algorithm used for testing, and DNA extraction methods used. We distribute this PT report to all participants, state laboratory directors, and program colleagues by request.

On October 5, 2015, a panel of five unknown dried blood spot (DBS) specimens was distributed to 32 laboratories in the United States and 35 laboratories in other countries to detect mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

PARTICIPANT RESULTS

This panel consisted of five DBS specimens (415C1, 415C2, 415C3, 415C4, and 415C5) prepared from adult CF patients, carriers, or unaffected individuals.

Evaluations are based on the genotype and clinical assessment of each specimen. Each clinical assessment counts as 10% and each identified allele counts as 5% of the assessment. If one or both alleles in a specimen are not part of a

laboratory's testing panel or would not be identified based on their screening algorithm, the specimen is considered not evaluated. Each participant is only graded on samples for which they can evaluate the alleles fully.

We received and processed data from 63 participants. Laboratories were asked to report the method(s) used in their testing and the alleles found for each specimen. One laboratory reported clinical assessments only and did not report any alleles.

For specimen 415C1, one laboratory reported an incorrect allele where both alleles should have been F508del (c.1521_1523delCTT). All laboratories were evaluated and all laboratories reported the correct clinical assessment.

For specimen 415C2, one laboratory reported an incorrect allele, S549N (c.1646G>A) and one laboratory did not detect a mutation causing an incorrect clinical assessment. Three methods detect the S492F (c.1475C>T) allele, resulting in the specimen being evaluated by the 9 participants that use these methods.

For specimen 415C3, all participants reported the correct clinical assessment and all reported alleles were correct.

For specimen 415C4, fifteen methods detected both the G542X (c.1624G>T) and the W1282X (c.3846G>A) alleles, resulting in the specimen being evaluated by the 54 participants that use these methods.

For specimen 415C5, all laboratories were evaluated and all reported alleles were correct. One laboratory reported an incorrect clinical assessment.

CDC/APHL

This program is cosponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL).

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The Newborn Screening Quality Assurance Program will ship next quarter's Cystic Fibrosis Mutation Detection PT specimens on January 11, 2016.

Please note that in order to receive an evaluation, you must use the current data report form and fill in all relevant information. This form can be downloaded from our website at http://www.cdc.gov/labstandards/nsqap_resources.html#QCReportForms

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NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

CYSTIC FIBROSIS MUTATION DETECTION SURVEY

QUARTER 4 –2015

TABLE 1. SPECIMEN CERTIFICATION

Specimen	Allele 1	Allele 2	Expected Clinical Assessment
415C1	F508del (c.1521_1523delCTT)	F508del (c.1521_1523delCTT)	2
415C2	S492F (c.1475C>T)	No mutations detected	2
415C3	No mutations detected	No mutations detected	1
415C4	G542X (c.1624G>T)	W1282X (c.3846G>A)	2
415C5	F508del (c.1521_1523delCTT)	No mutations detected	2

1 = Screen Negative (Normal)

2 = Screen Positive - 1 or 2 Mutations Detected

Alleles were determined or confirmed by CDC

TABLE 2. OVERALL FREQUENCY OF CLINICAL ASSESSMENTS

SPECIMEN ID	SCREEN NEGATIVE	SCREEN POSITIVE		NO CLINICAL ASSESSMENT REPORTED	NO DATA SUBMITTED	INCORRECT CLINICAL ASSESSMENTS**
		1 OR 2 MUTATIONS DETECTED				
C1	0	63		0	4	0
C2	55	8		0	4	1
C3	63	0		0	4	0
C4	3	60		0	4	0
C5	1	62		0	4	1

*Late results are maintained by NSQAP, but not included in evaluation statistics

**Methods vary widely based upon panel of mutations detected, the algorithm used for testing, and DNA extraction methods. These factors are considered in evaluation determination.

TABLE 3. OVERALL FREQUENCY OF REPORTED GENOTYPES

SPECIMEN ID	Allele	F508del (c.1521_1523delCTT)	S492F (c.1475C>T)	S549N (c.1646G>A)	G542X (c.1624G>T)	W1282X (c.3846G>A)	NO MUTATIONS DETECTED*	NO GENOTYPE REPORTED (Cell left blank)	INCORRECT GENOTYPE (by allele)
		C1	Allele 1	62					
C1	Allele 2	61					1	1	1
C2	Allele 1		6	1			55	1	1
C2	Allele 2						62	1	
C3	Allele 1						62	1	
C3	Allele 2						62	1	
C4	Allele 1				54	5	3	1	
C4	Allele 2				5	49	8	1	
C5	Allele 1	60					2	1	
C5	Allele 2	2					60	1	

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TABLE 4. PRIMARY METHODS

	Number of Laboratories
CF1 Hologic CF Inplex Molecular Test - ACMG	3
CF2 Hologic CF Inplex Molecular Test 40+4	20
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	7
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	1
CF7 Luminex Platform and Laboratory Developed Test	1
CF8 Elucigene Diagnostics CF4v2	1
CF10 Elucigene Diagnostics CF30	2
CF11 Elucigene Diagnostics CFEUv2	1
CF12 Abbott Molecular CF Genotyping Assay v3	4
CF15 Inno-LiPA Strips 17+19	2
CF16 Sequenom (MALDI-TOF Mass Spectrometry)	2
CF17 ViennaLab Diagnostics GmbH CF StripAssay	1
CF20 Allele-specific Oligonucleotide PCR	2
CF21 High Resolution Melt Technology	2
CF22 Real-time PCR Allelic Discrimination Assay (ie TaqMan)	1
CF23 In-house Amplification Refractory Mutation System	1
CF27 Amplification and Restriction Fragment Length Polymorphism Analysis (PCR-RFLP)	2
CF29 Sequencing	4
CF19 Other	6
No response	4

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TABLE 5. SECONDARY METHODS

	Number of Laboratories
CF1 Hologic CF Inplex Molecular Test - ACMG	1
CF2 Hologic CF Inplex Molecular Test 40+4	7
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	4
CF12 Abbott Molecular CF Genotyping Assay v3	3
CF14 Inno-LiPA Strip 19	1
CF15 Inno-LiPA Strips 17+19	1
CF16 Sequenom (MALDI-TOF Mass Spectrometry)	2
CF23 In-house Amplification Refractory Mutation System	1
CF25 PCR/Heteroduplex Analysis/Gel Electrophoresis	2
CF26 Capillary Electrophoresis	1
CF27 Amplification and Restriction Fragment Length Polymorphism Analysis (PCR-RFLP)	1
CF28 Amplification and Polyacrylamide Gel Electrophoresis (PCR-PAGE)	1
CF29 Sequencing	6
CF19 Other	4
No response	32

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TABLE 6. EXTRACTION METHODS

	Number of Laboratories
X1 Qiagen QIAamp spin columns (manual or robotic)	7
X2 Qiagen magnetic bead kit (EZ1 or BioSprint 96)	1
X3 Qiagen Generation DNA Purification & DNA Elution Solutions	22
X4 Sigma Aldrich Extract-N-Amp	2
X5 in-house alkaline lysis prep	6
X6 in-house boiling prep	6
X7 in-house lysis boiling prep	2
X19 Other	14
No response	7

This **NEWBORN SCREENING QUALITY ASSURANCE PROGRAM** report is an internal publication distributed to program participants and selected program colleagues. The laboratory quality assurance program is a project cosponsored by the **Centers for Disease Control and Prevention (CDC)** and the **Association of Public Health Laboratories**.

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