Newborn Screening Quality Assurance Program

Quarterly Report
Volume 12, No.1

Cystic Fibrosis DNA Mutation Detection Proficiency Testing Program (CFDNAPT)

2018 Quarter 1 March

Introduction

This report is the quarterly summary of all data reported within the specified data-reporting period for the Quarter 1, 2018 program for cystic fibrosis (CF) mutation detection for the Newborn Screening Quality Assurance Program (NSQAP). It is distributed to all participants, state laboratory directors, and program colleagues by request. The contents provide the certification profiles for the distributed specimens, the primary and secondary methods and the DNA extraction methods used by participants, the overall summary of reported alleles, and the overall summary of clinical assessments reported. An evaluation of reported data is attached to individual laboratory reports .

Certification of PT Specimens

The Quarter 1 panel consisted of five dried blood spot (DBS) specimens (118C1, 118C2, 118C3, 118C4, and 118C5) prepared from CF patients, carriers, or unaffected individuals. All mutations are characterized at CDC using Sanger sequencing and mutations are confirmed in DBS specimens using genotyping and next generation sequencing technologies. Prior to distribution, DNA was extracted from DBS samples with Qiagen Generation DNA Purification & DNA Elution Solutions (also sold as 5 Prime Easy PCR Solutions 1 & 2) and an in-house boiling prep method, and was run using Luminex Molecular Diagnostics xTAG CF 60 v2 to verify robust performance.

Table 1. Specimen Certification

Specimen	Allele 1	Allele 2	Clinical Assessment
118C1	No mutations detected	No mutations detected	1 (Screen Negative-Normal)
118C2	F508del (c.1521_1523delCTT)	R1066C (c.3196C>T)	2 (Screen Positive- 1 or 2 mutations)
118C3	G551D (c.1652G>A)	2789+5G>A (c.2657+5G>A)	2 (Screen Positive- 1 or 2 mutations)
118C4	3876delA (c.3744delA)	No mutations detected	2 (Screen Positive- 1 or 2 mutations)
118C5	F508del (c.1521_1523delCTT)	No mutations detected	2 (Screen Positive- 1 or 2 mutations)

1 = Screen Negative (Normal) 2 = Screen Positive - 1 or 2 Mutations Detected

Distribution of PT Specimens

On January 9, 2018, NSQAP distributed a panel of five unknown DBS specimens to 34 laboratories in the United States and 40 laboratories in other countries to detect mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

Participant Results

Data was received from 66 participants by the data reporting deadline. Participants tested specimens by the analytical schemes they routinely use. Reported data included method(s), mutation panel(s), screening algorithms, alleles found for each specimen and clinical assessments. If a method was not commercially available, the participant was asked to provide the mutation panel or regions sequenced in order for the submission to be accepted.

♦ Reported Method Data

Methods varied widely with regard to the panel of mutations detected, the algorithm used for testing, and the DNA extraction methods used. Tables 2 – 4 provide the frequencies for primary, secondary and extraction methods reported by participants.

Table 2. Frequency of Reported Primary Methods

Primary Method	# of Labs
CF1 GenMark Cystic Fibrosis Genotyping	4
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	14
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	10
CF6 Luminex Molecular Diagnostics xTAG CF 71 v2	1
CF7 Luminex Platform and Laboratory Developed Test	1
CF10 Elucigene Diagnostics CF30v2	3
CF11 Elucigene Diagnostics CF-EU2v1	5
CF15 Inno–LiPA Strips 17+19	2
CF16 Sequenom HerediT CF assay	1
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	1
CF18 ViennaLab Diagnostics GmbH CF StripAssay GER	5
CF20 Allele-specific Oligonucleotide PCR	2
CF21 High Resolution Melt Technology	1
CF22 Real-time PCR Allelic Discrimination Assay (i.e. TaqMan)	2
CF23 In-house Amplification Refractory Mutation System	1
CF26 Capillary Electrophoresis	3
CF27 Amplification and Restriction Fragment Length Polymorphism Analysis (PCR-RFLP)	1
CF29 Next Gen Sequencing - Illumina MiSeqDx 139 Variant Assay	2
CF30 Next Gen Sequencing - Multiplicom Molecular Diagnostics CFTR MASTR v2	1
CF32 All other gene sequencing protocols including Sanger and Next Gen	4
CF34 Devyser CFTR Core	1
CF99 Other	1

Table 3. Frequency of Reported Secondary Methods

Secondary Method	# of Labs
CF1 GenMark Cystic Fibrosis Genotyping	2
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	5
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	2
CF11 Elucigene Diagnostics CF-EU2v1	2
CF15 Inno–LiPA Strips 17+19	3
CF22 Real-time PCR Allelic Discrimination Assay (i.e. TaqMan)	1
CF25 PCR/ Heteroduplex Analysis/ Gel Electrophoresis	2
CF26 Capillary Electrophoresis	1
CF31 Next Gen Sequencing - Ion AmpliSeq CFTR Community Panel	1
CF32 All other gene sequencing protocols including Sanger and Next Gen	7
CF99 Other	3
No secondary method reported	37

Table 4. Frequency of Reported Extraction Methods

Extraction Method	# of Labs
X1 Qiagen QIAamp spin columns (manual or robotic)	6
X2 Qiagen magnetic bead kit (EZ1 or BioSprint 96)	2
X3 Qiagen Generation DNA Purification & DNA Elution Solutions	23
X4 Sigma Aldrich Extract-N-Amp	3
X5 in-house alkaline lysis prep	8
X6 in-house boiling prep	3
X7 in-house lysis boiling prep	2
X19 Other	19

♦ Allele Assessment Data

Table 5 provides the overall frequency of participant reported alleles for each specimen.

Table 5. Overall Frequency of Reported Alleles

Specimen	118C1		118C2		118C3		118C4		118C5	
Allele	1	2	1	2	1	2	1	2	1	2
F508del (c.1521_1523delCTT)			64	2					61	5
R1066C (c.3196C>T)				28						
G551D (c.1652G>A)					50	11				
2789+5G>A (c.2657+5G>A)					11	44				
3876delA (c.3744delA)							33	3		
3120+1G>A (c.2988+1G>A)							1			
No Mutations Detected	66	66	2	36	5	11	32	63	5	61
Allele Not Reported										
Incorrect Allele(s)							1			

♦ Clinical Assessment Data

All specimens were evaluated based on participants' specific method(s), mutation panel, and algorithm. Thus, the clinical assessments may vary between laboratories while still being correct. Table 6 provides the overall frequency of the participants' clinical assessments for each specimen.

Table 6. Overall Frequency of Clinical Assessments

Clinical Assessment	118C1	118C2	118C3	118C4	118C5
Screen Negative	66		5	29	
Screen Positive (1 or 2 Mutations Detected)		66	61	37	66
Clinical Assessment Not Reported					
Incorrect Clinical Assessment(s)				1	

Evaluations

Evaluations are based on the allele assessment and clinical assessment of each specimen. Each clinical assessment is worth 10% and each identified allele is worth 5% of the assessment. Since participants are graded according to their screening method(s), mutation panel, and algorithm, the clinical assessments may vary from laboratory to laboratory.

NSQAP received and processed data from 66 participants. One laboratory reported no data due to the Hologic recall and seven laboratories did not report data for this quarter.

Summary of Overall Evaluations for each Specimen

Specimen 118C1 – all submitted results had the correct clinical assessment of screen negative

Specimen 118C2 – all submitted results had the correct clinical assessment of screen positive; all reported alleles were correct based on the reported mutation panel or algorithm

Specimen 118C3 – 5 participants reported a clinical assessment of screen negative and 61 participants reported a clinical assessment of screen positive; all reported alleles were correct based on the reported mutation panel or algorithm

Specimen 118C4 – 29 participants reported a clinical assessment of screen negative and 37 participants reported a clinical assessment of screen positive; one participant reported an incorrect allele and clinical assessment based on their mutation panel or algorithm

Specimen 118C5 – all submitted results had the correct clinical assessment of screen positive; all reported alleles were correct based on the reported mutation panel or algorithm

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter's PT specimens for the CFDNAPT on April 3, 2018.

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The content of this report may also be located on our website at:
http://www.cdc.gov/labstandards/nsqap_reports.html

REPORT AUTHORIZATION

This report has been reviewed and authorized by Dr. Suzanne Cordovado, Laboratory Chief, Molecular Quality Improvement Program.

CONFIDENTIALITY STATEMENT

NSQAP participant information and evaluations are strictly confidential and shared only with individual participants, unless written authorization for release is received.

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NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

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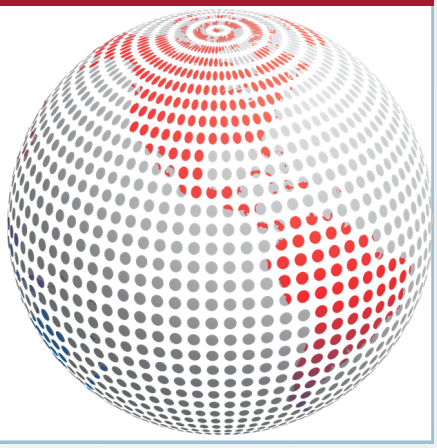

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