Newborn Screening Quality Assurance Program

Quarterly Report
Volume 11, No.2

Cystic Fibrosis DNA Mutation Detection Proficiency Testing Program (CFDNAPT)

2017 Quarter 2 May

Introduction

This report is the quarterly summary of all data reported within the specified data-reporting period for the Quarter 2, 2017 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 overall summary of clinical assessments reported, the overall summary of reported alleles, the primary and secondary methods used by participants, and the DNA extraction methods used by participants. An evaluation of your reported data is attached to this report.

Certification of PT Specimens

The Quarter 2 panel consisted of five dried blood spot (DBS) specimens (217C1, 217C2, 217C3, 217C4, and 217C5) prepared from adult 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 send out, DNA is 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
217C1	F508del (c.1521_1523delCTT)	621+1G>T (c.489+1G>T)	2 (Screen Positive- 1 or 2 mutations)
217C2	No mutations detected	No mutations detected	1 (Screen Negative-Normal)
217C3	S549N (c.1646G>A)	No mutations detected	2 (Screen Positive- 1 or 2 mutations)
217C4	F508del (c.1521_1523delCTT)	F508del (c.1521_1523delCTT)	2 (Screen Positive- 1 or 2 mutations)
217C5	F508del (c.1521_1523delCTT)	R1066C (c.3196C>T)	2 (Screen Positive- 1 or 2 mutations)

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

Distribution of PT Specimens

On April 3, 2017, NSQAP distributed a panel of five unknown DBS specimens to 33 laboratories in the United States and 48 laboratories in other countries to detect mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

Participant Results

Data was received from 68 participants by the data reporting deadline. Late results are maintained by NSQAP, but not included in evaluation statistics. Participants are required to survey specimens by the analytical schemes they routinely use. Reported data must include testing method(s), mutation panel(s), screening algorithms, alleles found for each specimen and clinical assessments. If a method is not commercially available, the participant must 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	3
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	15
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	10
CF7 Luminex Platform and Laboratory Developed Test	1
CF8 Elucigene Diagnostics CF4v2	1
CF10 Elucigene Diagnostics CF30v2	3
CF11 Elucigene Diagnostics CF-EU2v1	6
CF12 Abbott Molecular CF Genotyping Assay v3	1
CF15 Inno–LiPA Strips 17+19	2
CF16 Sequenom HerediT CF assay	1
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	2
CF18 ViennaLab Diagnostics GmbH CF StripAssay GER	5
CF20 Allele-specific Oligonucleotide PCR	2
CF21 High Resolution Melt Technology	2
CF22 Real-time PCR Allelic Discrimination Assay (i.e. TaqMan)	2
CF23 In-house Amplification Refractory Mutation System	1
CF26 Capillary Electrophoresis	2
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 NextGen	3
CF34 Devyser CFTR Core	1
CF99 Other	1

Table 3. Frequency of Reported Secondary Methods

Secondary Method	# of Labs
CF1 GenMark Cystic Fibrosis Genotyping	1
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	6
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	2
CF11 Elucigene Diagnostics CF-EU2v1	2
CF15 Inno–LiPA Strips 17+19	3
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	1
CF18 ViennaLab Diagnostics GmbH CF StripAssay, GER	1
CF25 PCR/ Heteroduplex Analysis/ Gel Electrophoresis	1
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 response	39

Table 4. Frequency of Reported Extraction Methods

Extraction Method	# of Labs
X1 Qiagen QIAamp spin columns (manual or robotic)	7
X2 Qiagen magnetic bead kit (EZ1 or BioSprint 96)	3
X3 Qiagen Generation DNA Purification & DNA Elution Solutions	23
X4 Sigma Aldrich Extract-N-Amp	1
X5 in-house alkaline lysis prep	7
X6 in-house boiling prep	5
X7 in-house lysis boiling prep	1
X19 Other	20
No response	1

♦ Genotype Data

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

Table 5. Overall Frequency of Reported Alleles

Specimen	217C1		217C2		217C3		217C4		217C5	
Allele	1	2	1	2	1	2	1	2	1	2
F508del (c.1521_1523delCTT)	34	34					68	67	63	5
621+1G>T (c.489+1G>T)	32	25								
S549N (c.1646G>A)					38	5				
R1066C (c.3196C>T)										26
3120+1G>A (c.2988+1G>A)					1					
G551D (c.1652G>A)						1				
S492F (c.1475C>T)					1					
I507del (c.1519_1521delATC)										1
No Mutations Detected	2	9	68	68	28	62		1	5	36
Incorrect Allele(s)					2	1		1		1

♦ Clinical Assessment Data

All specimens are evaluated for all participants based on their specific method, 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	217C1	217C2	217C3	217C4	217C5
Screen Negative		66	24		3
Screen Positive (1 or 2 Mutations Detected)	66		42	66	63
Clinical Assessment Not Reported	2	2	2	2	2
Incorrect Clinical Assessment(s)			2		3

Evaluations

Evaluations are based on the genotype 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 68 participants. One laboratory reported no data due to the Hologic recall and twelve laboratories did not report data for this quarter.

Summary of Overall Evaluations for each Specimen

Specimen 217C1 – all submitted results had the correct clinical assessment of screen positive and all reported alleles were correct

Specimen 217C2 – all submitted results had the correct clinical assessment of screen negative

Specimen 217C3 – 24 participants reported a clinical assessment of screen negative and 42 participants reported a clinical assessment of screen positive; two participants reported an incorrect clinical assessment and two participants reported incorrect allele(s) based in their mutation panel or algorithm

Specimen 217C4 – all submitted results had the correct clinical assessment of screen positive; one participant reported an incorrect allele

Specimen 217C5 – three participants reported a clinical assessment of screen negative and 63 participants reported a clinical assessment of screen positive; three participants reported an incorrect clinical assessment and one participant reported an incorrect allele

Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter's PT specimens for the CFDNAPT on July 10, 2017

Acknowledgements

We would like to thank Philip Farrell, M.D., Ph.D. (University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin), Martin Kharrazi, Ph.D. (California Department of Public Health, Richmond, California), Charlene Sacramento (Sequoia Foundation, La Jolla, California) and all the CF Care Clinics for their collaboration and efforts in this project. We would also like to thank the anonymous blood donors for participating. Without their contributions, this program would not be possible.

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

This program is co-sponsored by the Centers for Disease Control and Prevention (CDC) and The Association of Public Health Laboratories (APHL)

NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

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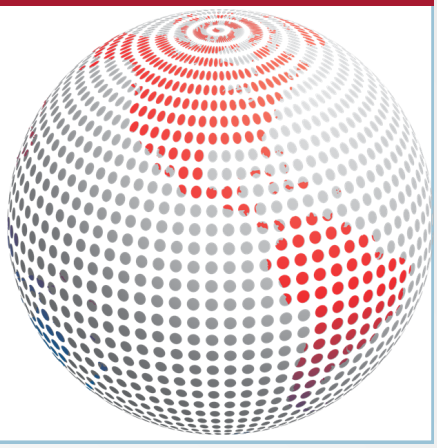

This *NEWBORN SCREENING QUALITY ASSURANCE PROGRAM* report is an internal publication distributed to program participants and selected program colleagues. The laboratory quality assurance program is a project cosponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public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

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