

Cystic Fibrosis DNA Mutation Detection Proficiency Testing Program (CFDNAPT)

2017 Quarter 4 November

Introduction

This report is the quarterly summary of all data reported within the specified data-reporting period for the Quarter 4, 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 reported data is attached to individual laboratory reports.

Certification of PT Specimens

The Quarter 4 panel consisted of five dried blood spot (DBS) specimens (417C1, 417C2, 417C3, 417C4, and 417C5) 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 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
417C1	No mutations detected	No mutations detected	1 (Screen Negative-Normal)
417C2	L206W (c.617T>G)	No mutations detected	2 (Screen Positive- 1 or 2 mutations)
417C3	F508del (c.1521_1523delCTT)	R553X (c.1657C>T)	2 (Screen Positive- 1 or 2 mutations)
417C4	F508del (c.1521_1523delCTT)	3905insT (c.3773dupT)	2 (Screen Positive- 1 or 2 mutations)
417C5	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 October 2, 2017, 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 69 participants by the data reporting deadline. Late results will no longer be accepted for any reason. 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	15
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	9
CF7 Luminex Platform and Laboratory Developed Test	1
CF8 Elucigene Diagnostics CF4v2	1
CF10 Elucigene Diagnostics CF30v2	3
CF11 Elucigene Diagnostics CF-EU2v1	5
CF12 Abbott Molecular CF Genotyping Assay v3	1
CF15 Inno-LiPA Strips 17+19	3
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	2
CF22 Real-time PCR Allelic Discrimination Assay (i.e. TaqMan)	2
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	2
CF32 All other gene sequencing protocols including Sanger and Next Gen	5
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	3
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
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	2
No secondary method reported	38

Table 4. Frequency of Reported Extraction Methods

Extraction Method	# of Labs
X1 Qiagen QIAamp spin columns (manual or robotic)	8
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	3
X5 in-house alkaline lysis prep	9
X6 in-house boiling prep	2
X7 in-house lysis boiling prep	2
X19 Other	19

◆ Genotype Data

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

Table 5. Overall Frequency of Reported Alleles

Specimen	417C1		417C2		417C3		417C4		417C5	
	1	2	1	2	1	2	1	2	1	2
L206W (c.617T>G)			21	2						
F508del (c.1521_1523delCTT)					66	3	65	4	67	1
R553X (c.1657C>T)					2	61				
3905insT (c.3773dupT)							2	51		
F311del (c.933_935delCTT)									1	
No Mutations Detected	69	69	48	67	1	5	2	14	1	67
Allele Not Reported										1
Incorrect Allele(s)								1	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	417C1	417C2	417C3	417C4	417C5
Screen Negative	69	46			
Screen Positive (1 or 2 Mutations Detected)		23	69	69	69
Clinical Assessment Not Reported					
Incorrect Clinical Assessment(s)					

◆ 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 69 participants. One laboratory reported no data due to the Hologic recall and four laboratories did not report data for this quarter.

◆ Summary of Overall Evaluations for each Specimen

Specimen 417C1 – all submitted results had the correct clinical assessment of screen negative

Specimen 417C2 – 46 participants reported a clinical assessment of screen negative and 23 participants reported a clinical assessment of screen positive; all reported alleles were correct based on the reported mutation panel or algorithm

Specimen 417C3 – 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 417C4 – all submitted results had the correct clinical assessment of screen positive; one participant reported an incorrect allele based on their mutation panel or algorithm

Specimen 417C5 – all submitted results had the correct clinical assessment of screen positive; one participant reported an incorrect allele based on their mutation panel or algorithm and one participant did not report an allele

◆ Future Shipments

The Newborn Screening Quality Assurance Program will ship next quarter's PT specimens for the CFDNAPT on January 9, 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

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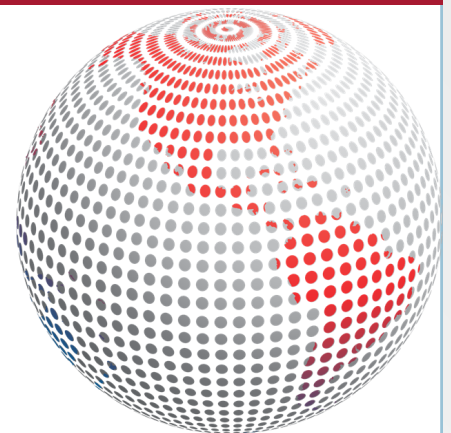
NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

Direct inquiries to:

Centers for Disease Control and Prevention
4770 Buford Highway NE, MS/F24
Atlanta, GA 30341-3724
Phone: 770-488-4048 Email: scordovado@cdc.gov

Editors:

Suzanne Cordovado
Miyono Hendrix
Joanne Mei
Irene Williams



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CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) ATLANTA, GA 30341

Director

Brenda Fitzgerald, M.D.

Director

National Center for Environmental Health

Patrick Breysse, Ph.D.

Director

Division of Laboratory Sciences

James L. Pirkle, M.D., Ph.D.

Chief

Newborn Screening and Molecular Biology Branch

Carla Cuthbert, Ph.D.

Contributors:

Suzanne Cordovado, Ph.D.

Zachery Detwiler

Katherine Duneman

Christopher Greene, Ph.D.

Laura Hancock

Miyono Hendrix

Deborah Koontz, Ph.D.

Thai Le

Joanne Mej, Ph.D.

Stanimila Nikolova, Ph.D.

Konstantinos Petritis, Ph.D.

Irene Williams

Sherri Zobel

Production:

Sarah Brown

Chloe Collins

Kimberly Coulter

Kizzy Stewart

ASSOCIATION OF PUBLIC HEALTH LABORATORIES SILVER SPRING, MD 20910

President

Ewa King, PhD

Chairman, Newborn Screening and Genetics in Public Health Committee

Michele Caggana, Sc.D., FACMG

Chairman, Newborn Screening Quality Assurance Quality Control Subcommittee

Patricia R. Hunt, B.A. and Joseph Orsini, Ph.D.

Chairman, Newborn Screening Molecular Subcommittee

Rachel Lee, Ph.D.

INQUIRIES TO:

Suzanne K. Cordovado, Editor • Centers for Disease Control and Prevention (CDC) • Newborn Screening Quality Assurance Program

Mailstop F-24 • 4770 Buford Highway, N.E. • Atlanta, GA 30341-3724

Phone (770) 488-4582 • NSQAPDMT@cdc.gov

E-mail: SCordovado@cdc.gov