

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

Cystic Fibrosis Mutation Detection
Quarterly Report

Volume 10, No. 2

May 2016

INTRODUCTION

This report is the quarterly summary of all data reported within the specified data-reporting period for the Quarter 2, 2016 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 overall summary of clinical assessments reported, the overall summary of reported alleles, 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 April 4, 2016, a panel of five unknown dried blood spot (DBS) specimens was distributed to 33 laboratories in the United States and 37 laboratories in other countries to detect mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. All specimens are evaluated for all participants based on their specific method, panel of mutations, and algorithm. Thus, the clinical assessments may vary between laboratories while still being correct.

PARTICIPANT RESULTS

This panel consisted of five DBS specimens (216C1, 216C2, 216C3, 216C4, and 216C5) prepared from adult CF patients, carriers, or unaffected individuals.

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) and algorithm, the clinical assessments may vary from laboratory to laboratory.

We received and processed data from 54 participants. Laboratories were asked to report their testing method(s), mutation panel(s), screening algorithm, the alleles found for each specimen and the clinical assessment. In March 2016, Hologic announced a voluntary recall of the InPlex Cystic Fibrosis ASR Cards and InPlex Cystic Fibrosis Molecular Test Kits. Thirteen laboratories reported limited or no data due to the recall. Four laboratories did not report data for this quarter.

Summary of participant results:

- Specimen 216C1 – all submitted results had the correct clinical assessment of screen negative
- Specimen 216C2 – all submitted results had the correct clinical assessment of screen positive; one participant reported an allele that was incorrect
- Specimen 216C3 – 50 participants reported a clinical assessment of screen negative and 4 participants reported a clinical assessment of screen positive; one participant did not identify the 2105-2117del13insAGAAA (c.1973_1985del13insAGAAA) mutation that was part of their reported panel resulting in an incorrect clinical assessment
- Specimen 216C4 – 18 participants reported a clinical assessment of screen negative and 35 participants reported a clinical assessment of screen positive; two participants did not identify the 3905insT (c.3773_3774insT) mutation that was part of their reported panel resulting in incorrect clinical assessments

CDC/APHL

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

Direct inquiries to:
Centers for Disease Control and Prevention (CDC)
4770 Buford Highway, NE, MS/F24
Atlanta, GA 30341-3724

Phone: 770-488-4048
FAX: 770-488-4255
E-mail: SCordovado@cdc.gov

Editor: Suzanne Cordovado
Miyono Hendrix
Irene Williams
Joanne Mei



- Specimen 216C5 – one participant reported a clinical assessment of screen negative, 51 participants reported a clinical assessment of screen positive and one participant did not report a clinical assessment; one participant did not identify the F508del (c.1521_1523delCTT) mutation that was part of their reported panel resulting in an incorrect clinical assessment

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

Please note that beginning in Q3 2016, participants must use the current data report form and fill in all relevant information in order to receive an evaluation. This form can be downloaded from our website at http://www.cdc.gov/lab-standards/nsqap_resources.html#QCReportForms.

ACKNOWLEDGMENTS

We would like to thank Philip Farrell, M.D., Ph.D. (University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin), Marty Kharrazi, Ph.D. (California Department of Public Health, Richmond, California), Charlene Sacramento (Sequoia Foundation, La Jolla, California) and all the collection centers 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.

NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

CYSTIC FIBROSIS MUTATION DETECTION SURVEY

QUARTER 2 –2016

TABLE 1. SPECIMEN CERTIFICATION

Specimen	Allele 1	Allele 2	Expected Clinical Assessment
216C1	No mutations detected	No mutations detected	1
216C2	F508del (c.1521_1523delCTT)	F508del (c.1521_1523delCTT)	2
216C3	2105-2117del13insAGAAA (c.1973_1985del13insAGAAA)	No mutations detected	2
216C4	3905insT (c.3773_3774insT)	No mutations detected	2
216C5	F508del (c.1521_1523delCTT)	711+1G>T (c.579+1G>T)	2

1 = Screen Negative (Normal)

2 = Screen Positive - 1 or 2 Mutations Detected

Alleles were determined or confirmed by CDC

TABLE 2. OVERALL REPORTED CLINICAL ASSESSMENTS

SPECIMEN ID	SCREEN NEGATIVE	SCREEN POSITIVE 1 OR 2 MUTATIONS DETECTED	NO CLINICAL ASSESSMENT REPORTED	NO DATA SUBMITTED	INCORRECT CLINICAL ASSESSMENTS	HOLOGIC RECALL
216C1	54	0	0	4	0	12
216C2	0	53	0	4	0	13
216C3	50	4	0	4	1	12
216C4	18	35	0	4	2	13
216C5	1	51	1	4	1	13

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

Screening method(s) and algorithms used by participants differ, thus the clinical assessments may vary from laboratory to laboratory.

TABLE 3. OVERALL REPORTED ALLELES

	F508del (c.1521_1523delCTT)	2105-2117del13insAGAAA (c.1973_1985del13insAGAAA)	3905insT (c.3773_3774insT)	711+1G>T (c.579+1G>T)	NO MUTATIONS DETECTED	HOLOGIC RECALL	NO MUTATIONS REPORTED	INCORRECT ALLELES
216C1	Allele 1				54	12	4	
	Allele 2				54	12	4	
216C2	Allele 1	53				13	4	
	Allele 2	52			1	13	4	1
216C3	Allele 1		4		50	12	4	1
	Allele 2				54	12	4	
216C4	Allele 1		32		21	13	4	2
	Allele 2		3		50	13	4	
216C5	Allele 1	39		12	2	13	4	1
	Allele 2	13		27	13	13	4	

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CYSTIC FIBROSIS MUTATION DETECTION SURVEY

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TABLE 4. PRIMARY METHODS

	Number of Laboratories
CF2 Hologic CF Inplex Molecular Test 40+4	6
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	8
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	4
CF7 Luminex Platform and Laboratory Developed Test	1
CF8 Elucigene Diagnostics CF4v2	1
CF10 Elucigene Diagnostics CF30v2	3
CF11 Elucigene Diagnostics CF-EU2v1	3
CF12 Abbott Molecular CF Genotyping Assay v3	3
CF15 Inno-LiPA Strips 17+19	2
CF16 Sequenom HerediT CF assay	1
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	3
CF18 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)	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	1
CF30 Next Gen Sequencing - Multiplicom Molecular Diagnostics CFTR MASTR v2	1
CF32 All other gene sequencing protocols including Sanger and Next Gen	3
CF99 Other	3
No data was reported due to Hologic Recall	12
No response	4

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TABLE 5. SECONDARY METHODS

	Number of Laboratories
CF2 Hologic CF Inplex Molecular Test 40+4	2
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	4
CF12 Abbott Molecular CF Genotyping Assay v3	2
CF14 Inno-LiPA Strip 19	1
CF15 Inno-LiPA Strips 17+19	1
CF16 Sequenom HerediT CF assay	1
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	1
CF23 In-house Amplification Refractory Mutation System	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	10
CF99 Other	4
No response	39

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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)	2
X3 Qiagen Generation DNA Purification & DNA Elution Solutions	17
X4 Sigma Aldrich Extract-N-Amp	3
X5 in-house alkaline lysis prep	6
X6 in-house boiling prep	4
X7 in-house lysis boiling prep	1
X19 Other	12
No data was reported due to Hologic Recall	12
No response	6

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**.

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

Director

Thomas R. Frieden, M.D., M.P.H.

Director

National Center for Environmental Health

Patrick Breyse, 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
Thai Le
Deborah Koontz, Ph.D.
Joanne Mei, Ph.D.
Stanimila Nikolova, Ph.D.
Dino Romero
Irene Williams
Sherri Zobel

Production: Sarah Brown
Kimberly Coulter
Chinh Nguyen
LoNeka Shockley

ASSOCIATION OF PUBLIC HEALTH LABORATORIES
SILVER SPRING, MD 20910

President

Judith C. Lovchik, Ph.D., D(ABMM)

Chairman, Newborn Screening and Genetics in Public Health Committee

Susan M. Tanksley, Ph.D.

Chairman, Newborn Screening Quality Assurance Quality Control Subcommittee

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

Chairman, Newborn Screening Molecular Subcommittee

Michele Caggana, Sc.D., FACMG



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 • FAX (770) 488-4255 • E-mail: SCordovado@cdc.gov