

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

Cystic Fibrosis Mutation Detection
Quarterly Report

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INTRODUCTION

This report is the quarterly summary of all data reported within the specified data-reporting period for the Quarter 1, 2015 program for cystic fibrosis (CF) mutation detection. The attached tables provide the certification profiles for the distributed specimens, the verification of your reported data, the summary of reported genotypes, and the frequency distribution summary for expected interpretations. We distribute this PT report to all participants, state laboratory directors, and program colleagues by request.

On January 12, 2015 a panel of five unknown dried blood spot (DBS) specimens was distributed to 32 laboratories in the United States and 32 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 prepared from adult CF patients, carriers, or unaffected individuals (specimens 115C1, 115C2, 115C3, 115C4, and 115C5).

Evaluations are based on the genotype and clinical assessment of each specimen. Each clinical assessment counts as 10% and each allele counts as 5% of the assessment. Expected genotypes may differ by participant because of the panel of mutations, screening algorithm, or method used. In these cases, an answer of “no mutation detected” is acceptable and participants will receive a 100% satisfactory assessment.

We processed data from 62 participants. Laboratories were asked to report the method used and the genotype for each

specimen. Methods varied widely with regard to the panel of mutations detected, the algorithm used for testing, and DNA extraction methods used. These methods and the number of laboratories that use them are shown in tables included in this report.

The genotype for specimen 115C1 was F508del/No mutations detected. In addition, the specimen contained the polymorphism F508C which impacts the same amino acid as F508del. Some commercially available assays identify the F508C polymorphism so F508del/F508C is an acceptable answer. Laboratories that use a 2 probe hybridization method to detect both F508del and normal sequence or allele-specific oligonucleotide PCR are not able to discriminate between the normal sequence and the F508C variant and are evaluated as having an incorrect answer genotype.

One laboratory did not report any genotypes and one laboratory did not report any clinical assessments. For specimen 115C1, two laboratories reported an incorrect clinical assessment, and four laboratories reported an incorrect genotype. One laboratory reported an incorrect genotype for specimen 115C2 and two laboratories reported an incorrect genotype for specimen 115C3. One laboratory did not report a clinical assessment and one laboratory reported a sample failure for specimen 115C4. For specimen 115C5, two laboratories did not report a clinical assessment, one laboratory reported an incorrect clinical assessment, and one laboratory reported an incorrect genotype and clinical assessment.

Five methods can detect the R1158X (c.3472C>T) mutation included in specimen 115C5. Consequently, the specimen was evaluated for the eight participants that use one of these methods. The Newborn Screening Quality Assurance

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/F43
Atlanta, GA 30341-3724

Phone: 770-488-7828
FAX: 770-488-4255
E-mail: MEarley@cdc.gov

Editor: Marie Earley
Irene Williams
Joanne Mei



Program will ship next quarter's Cystic Fibrosis Mutation Detection PT specimens on April 6, 2015.

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

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CYSTIC FIBROSIS MUTATION DETECTION SURVEY

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TABLE 1. SPECIMEN CERTIFICATION

Specimen	Allele 1	Allele 2	Expected Clinical Assessment
115C1	F508del (c.1521_1523delCTT)	F508C (c.1523C>G)* or No mutations detected	2
115C2	F508del (c.1521_1523delCTT)	F508del (c.1521_1523delCTT)	2
115C3	G542X (c.1624G>T)	3849+10kB C>T (c.3717+12191C>T)	2
115C4	No mutations detected	No mutations detected	1
115C5	R1158X (c.3472C>T)	No mutations detected	2

1 = screen negative (normal)

2 = 1 or 2 mutations detected

*F508C is a polymorphism found in the same position as F508del and could interfere with an assay. Some commercial kits may provide reagents for reflex testing or may include F508C as part of its normal process to eliminate the need for reflex testing.

Allele information provided for each specimen was confirmed or determined by CDC.

TABLE 2. OVERALL FREQUENCY OF CLINICAL ASSESSMENTS

SPECIMEN ID	SCREEN	SCREEN	NO CLINICAL	NO DATA	INCORRECT
	NEGATIVE	POSITIVE 1 OR 2 MUTATIONS DETECTED	ASSESSMENT REPORTED	SUBMITTED	CLINICAL ASSESSMENTS**
115C1	2	59	1	2	2
115C2	0	61	1	2	0
115C3	3	58	1	2	0
115C4	59	0	3	2	0
115C5	52	7	3	2	2

*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

		F508del	F508C	G542X	3849+10kB C>T	R1158X	NO MUTATIONS DETECTED*	NO GENOTYPE REPORTED (Cell left blank)	INCORRECT GENOTYPE (by allele)	INCORRECT CLINICAL ASSESSMENTS**
115C1†	Allele 1	61								
	Allele 2	4	16				41	1	4	2
115C2	Allele 1	62								
	Allele 2	61					1	1	1	0
115C3	Allele 1			57						
	Allele 2				53		4	1	2	0
115C4	Allele 1						59			
	Allele 2						59	2	0	0
115C5	Allele 1					8				
	Allele 2						61	1	1	2

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

**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.

†Four laboratories reported an incorrect genotype of F508del/F508del.

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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	19
CF3 Luminex Molecular Diagnostics xTAG CF - ACMG only	1
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	7
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	1
CF6 Luminex Molecular Diagnostics xTAG CF 71 v2	1
CF7 Luminex Platform and Laboratory Developed Test	1
CF8 Hologic Gen-Probe Elucigene CF4v2	1
CF10 Hologic Gen-Probe Elucigene CF30	2
CF11 Hologic Gen-Probe Elucigene CFEUv1	3
CF12 Abbott Molecular CF Genotyping Assay v3	4
CF14 Innogenetics Inno-LiPA Strip 19	1
CF15 Innogenetics Inno-LiPA Strips 17+19	2
CF16 Sequenom (MALDI-TOF Mass Spectrometry)	4
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	1
CF19 Other	2
No response	3

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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	4
CF14 Innogenetics Inno-LiPA Strip 19	1
CF15 Innogenetics Inno-LiPA Strips 17+19	3
CF16 Sequenom (MALDI-TOF Mass Spectrometry)	3
CF17 ViennaLab Diagnostics GmbH CF StripAssay	1
CF25 PCR/Heteroduplex Analysis/Gel Electrophoresis	1
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	5
CF19 Other	3
No response	28

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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	20
X4 Sigma Aldrich Extract-N-Amp	1
X5 in-house alkaline lysis prep	6
X6 in-house MeOH boiling prep	5
X7 in-house lysis boiling prep	2
X19 Other	14
No response	9

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:

Barbara W. Adam	Patrick Pickens
Suzanne Cordovado, Ph.D.	Kelsey Sheard
Paul Dantonio	Jennifer Taylor, Ph.D.
Victor R. De Jesus, Ph.D.	Robert Vogt, Ph.D.
Zachary Detwiler	Irene Williams
Marie C. Earley, Ph.D.	Golriz Yazdanpanah
Sharon Flores	Hui Zhou, Ph.D.
David Foreman	Sherri Zobel
Stephanie Foster	
Travis Gilliland	
Christopher Greene, Ph.D.	
Elizabeth M. Hall	
Laura Hancock	
Christopher Haynes, Ph.D.	
Miyono Hendrix	
Sarah Klass	
Deborah Koontz, Ph.D.	
Francis Lee, Ph.D.	
Lixia Li, Ph.D.	
Timothy Lim, Ph.D.	
Daniel Mandel, Ph.D.	
Joanne Mei, Ph.D.	
Stanimila Nikolova, Ph.D.	

Production:

Sarah Brown
Iris Landers
Felicia Manning
LoNeka Shockley

ASSOCIATION OF PUBLIC HEALTH LABORATORIES
SILVER SPRING, MD 20910

President

Dan Rice, DrPH, MS.

Chairman, Newborn Screening and Genetics in Public Health Committee

Susan M. Tanksley, Ph.D.

Chairman, Newborn Screening Quality Assurance Quality Control Subcommittee

Patrick Hopkins, B.S.



INQUIRIES TO:

Marie Earley, Editor • *Centers for Disease Control and Prevention (CDC)*
Newborn Screening Quality Assurance Program • *Mailstop F-43*
4770 Buford Highway, N.E. • *Atlanta, GA 30341-3724*
Phone (770) 488-4582 • *FAX (770) 488-4255* • *E-mail: MEarley@cdc.gov*