

Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

Cystic Fibrosis Mutation Detection Quarterly Report

Volume 7, No. 1

February 2013

INTRODUCTION

This report is the quarterly summary of all data reported within the specified data-reporting period for the Quarter 1, 2013 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 7, 2013 a panel of five unknown dried-blood-spot (DBS) specimens was distributed to 33 laboratories in the United States and 33 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 113C1, 113C2, 113C3, 113C4, and 113C5).

Evaluations are based on the clinical assessment of each specimen. Expected genotypes may differ by participant because of the panel of mutations tested. In these cases, an answer of “no mutation detected” is acceptable. A specimen is considered not evaluated when both of the expected mutations are not detected by the laboratory’s method.

We processed data from 58 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 and the algorithm used for testing. The specific methods and the number of laboratories that

use them are shown in the Laboratory Methods Table. Some laboratories screen specimens for a limited number of mutations and if a mutation was present, continue testing with an expanded panel. Laboratories were not asked to report the maximum number of mutations that could be detected.

One of the mutations in specimen 113C1 was 1154insTC. This mutation is only detected by sequencing or by specific laboratory developed tests so the specimen was still considered evaluated. One incorrect clinical assessment was reported for specimen 113C3. One of the mutations in specimen 113C3 was 3272-26A->G (c.3140-26A->G). This mutation is not detected by 97% of the laboratories in the United States but is commonly detected in some international laboratories so the designation of “Not Evaluated” was applied. No clinical assessment was reported by one laboratory for specimens 113C4 and 113C5. The Newborn Screening Quality Assurance Program will ship next quarter’s Cystic Fibrosis Mutation Detection PT specimens on April 1, 2013.

ACKNOWLEDGMENTS

We would like to thank Philip Farrell, M.D., Ph.D. (University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin) and 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.

New Evaluation Criteria for NSQAP’s Cystic Fibrosis Mutation Detection Proficiency Testing Program (starting July, 2013):

1. Clinical Assessments include Screen Positive (1 or 2

CDC/APHL

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

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mutations) or Screen Negative (0 mutations). Sample failure is no longer an option.

2. A specimen is considered not evaluated if both mutations are not on the method panel used by a laboratory.

Evaluations are based on both clinical assessment and the genotype reported. If a laboratory misses one or both mutations that are on their mutation detection panel, it will be considered a misclassification regardless of the clinical assessment.

To receive a 100% satisfactory evaluation, all 10 alleles for the 5 specimens and their corresponding clinical assessments must be correct based on the laboratory's detection panel.

For example, if a specimen is certified as G542X / F508del compound heterozygote, Screen Positive and a laboratory uses a method that detects both mutations yet only reports F508del and a clinical assessment of Screen Positive, the evaluation is reduced by 10%. In the case of a laboratory that tests for F508del only and reports F508del and a clinical assessment of Screen Positive, the evaluation is considered 100% satisfactory. When the clinical assessment is incorrect, it will be evaluated as incorrect regardless of the genotype.

Although not required for evaluation, we ask for your extraction method (commercial, in-house lyses, etc.). We are collecting this data because DNA extraction from dried-blood spots is not standardized and can be a source of problems in the downstream assay.

NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

CYSTIC FIBROSIS MUTATION DETECTION SURVEY

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SPECIMEN CERTIFICATION

Specimen	Allele 1	Allele 2	Expected Clinical Assessment
113C1	1717-1G->A (c.1585-1G->A)	1154insTC (c.1022_1023insTC)	2
113C2	F508del (p.Phe508del)	F508del (p.Phe508del)	2
113C3	F508del (p.Phe508del)	3272-26A->G (c.3140-26A->G)	2
113C4	Wild type	Wild type	1
113C5	Wild type	Wild type	1

1 = screen negative (normal)

2 = 1 or 2 mutations detected

Alleles were determined/confirmed by CDC and/or were included with the samples from the provider.

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CYSTIC FIBROSIS MUTATION DETECTION SURVEY
QUARTER 1 - FEBRUARY 2013
FREQUENCY OF REPORTED CLINICAL ASSESSMENTS

Specimen	Screen Negative (Normal)	Screen Positive (1 or 2 mutations detected) run
113C1	7	51
113C2	0	58
113C3	2	56
113C4*	57	0
113C5*	57	0

*One laboratory did not report any clinical assessments.

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INCORRECT ASSESSMENTS AND SPECIMENS NOT EVALUATED

Specimen	Incorrect	Not Evaluated*
113C1	0	7
113C2	0	0
113C3	1	46
113C4	0	1
113C5	0	1

*Includes sample failures, no reported clinical assessments, and when both mutations are not part of the laboratory's mutation panel.

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CYSTIC FIBROSIS MUTATION DETECTION SURVEY

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LABORATORY METHODS

Primary Method	Number of Laboratories
CF1 Hologic CF Inplex Molecular Test - ACMG	5
CF2 Hologic CF Inplex Molecular Test 40+4	17
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	8
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	2
CF10 Hologic Gen-Probe Elucigene CF30	3
CF12 Abbott Molecular CF Genotyping Assay v3	4
CF15 Innogenetics Inno-LiPA Strips 17+19	4
CF16 Sequenom (MALDI-TOF Mass Spectrometry)	1
CF17 ViennaLab Diagnostics GmbH CF StripAssay	1
CF20 Allele-specific Oligonucleotide PCR	1
CF22 Real-time PCR Allelic Discrimination Assay (ie TaqMan)	1
CF23 In-house Amplification Refractory Mutation System	1
CF25 PCR/Heteroduplex Analysis/Gel Electrophoresis	1
CF26 Capillary Electrophoresis	1
CF28 Amplification and Polyacrylamide Gel Electrophoresis (PCR-PAGE)	1
CF29 Sequencing	2
CF19 Other	2

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LABORATORY METHODS

Secondary Method	Number of Laboratories
CF1 Hologic CF Inplex Molecular Test - ACMG	1
CF2 Hologic CF Inplex Molecular Test 40+4	9
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	3
CF9 Hologic Gen-Probe Elucigene CF29v2	1
CF11 Hologic Gen-Probe Elucigene CFEUv1	1
CF12 Abbott Molecular CF Genotyping Assay v3	1
CF14 Innogenetics Inno-LiPA Strip 19	1
CF16 Sequenom (MALDI-TOF Mass Spectrometry)	1
CF21 High Resolution Melt Technology	1
CF25 PCR/Heteroduplex Analysis/Gel Electrophoresis	1
CF28 Amplification and Polyacrylamide Gel Electrophoresis (PCR-PAGE)	1
CF29 Sequencing	6
CF19 Other	5

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LABORATORY METHODS

Extraction Method	Number of Laboratories
X1 Qiagen QIAamp spin columns (manual or robotic)	2
X2 Qiagen magnetic bead kit (EZ1 or BioSprint 96)	1
X3 Qiagen Generation DNA Purification & DNA Elution Solutions	22
X4 Sigma Aldrich Extract-N-Amp	1
X5 in-house alkaline lysis prep	4
X6 in-house boiling prep	7
X19 Other	8
No response	13

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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