



Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

Cystic Fibrosis Mutation Detection Quarterly Report

Volume 3, No. 4

November 2009

INTRODUCTION

We initiated a proficiency testing (PT) program for cystic fibrosis (CF) mutation detection. This report is the quarterly summary of all data reported within the specified data-reporting period for Quarter 4, 2009. 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 distributions summary for expected interpretations. We distribute this PT report to all participants, state laboratory directors, and program colleagues by request.

On October 5, 2009, a panel of five unknown dried-blood-spot (DBS) specimens was distributed to 24 laboratories in the United States and 21 laboratories in other countries to detect mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

PARTICIPANT RESULTS

We distributed one type of DBS specimens in this panel. Five specimens were prepared from adult CF patients (specimens 49C1, 49C2, 49C3, 49C4, and 49C5).

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 one or both of the expected mutations is not detected by the laboratory's method or if the specimen cannot be assayed (sample failure).

We processed data from 43 participants. Laboratories were asked to report the genotype. Methods varied widely with regard to the panel of mutations detected and the algorithm used for testing. Thirteen used Third Wave Technologies Invader assay, 8 used Luminex Molecular Diagnostics (Tm Biosciences) Tag-It kit, 4 laboratories used Tepnel

Diagnostics Elucigene Assays, 3 used an amplification/gel electrophoresis assay, 2 used an in-house TaqMan Allelic Discrimination assay, 3 used Asuragen's Signature CF 2.0 assay, 1 used an Abbott Laboratories method, 1 used Innogenetics Inno-Lipa assay, 1 used an in-house PCR followed by restriction fragment length polymorphism analysis method, 1 used an in-house multi-plex PCR-heteroduplex and restriction enzyme method, 1 used a home-brew method, 1 used an in-house single nucleotide polymorphism assay, 2 used sequencing, 1 used allele-specific oligonucleotide PCR, 1 used in-house PCR with high resolution melt analysis, 1 used a PCR/heteroduplex analysis, 1 used an in-house allele-specific hybridization method, and 1 used Matrix Assisted Laser Desorption /Ionization- Time Of Flight (MALDI-TOF) mass spectrometry. Some laboratories used more than one method for their screening. One laboratory screened specimens for 4 mutations and if a mutation was present, continued testing with an expanded panel. The smallest panel consisted of 3 mutations. Laboratories were not asked to report the maximum number of mutations that could be detected. One incorrect clinical assessment was reported for Specimen 49C1 and one incorrect clinical assessment was reported for Specimen 49C3. No sample failures were reported. The Newborn Screening Quality Assurance Program will ship next quarter's Cystic Fibrosis Mutation Detection PT specimens on January 11, 2010. ❖

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 blood donors for participating. Without their contributions, this program would not be possible. ❖

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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NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

CYSTIC FIBROSIS MUTATION DETECTION SURVEY

QUARTER 4 – NOVEMBER 2009

LAB XXX

DATA VERIFICATION

Specimen Number	Allele 1	Allele 2	Clinical Assessment
49C1			
49C2			
49C3			
49C4			
49C5			

Reviewer's Comments

EVALUATION:

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FREQUENCY OF REPORTED CLINICAL ASSESSMENTS

Specimen	Screen Negative (Normal)	Likely Cystic Fibrosis Positive	Likely Cystic Fibrosis Carrier	Sample Failure
49C1	12	0	31	0
49C2	43	0	0	0
49C3	4	31	8	0
49C4	0	43	0	0
49C5	43	0	0	0

INCORRECT ASSESSMENTS AND SPECIMENS NOT EVALUATED

Specimen	Incorrect Assessment	Not Evaluated
49C1	1	0
49C2	0	0
49C3	1	11
49C4	0	0
49C5	0	0

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LABORATORY METHODS

Method	Number of Laboratories
Hologic (Third Wave Technologies) Invader Assay	13
Luminex Molecular Diagnostics (Tm Biosciences) Tag-It	8
Tepnel Diagnostics Elucigene Assay (CF-29, CF-30, or CF-4, CF-EU1)	4
Amplification / gel electrophoresis	3*
Asuragen Signature CF 2.0	3
In-house TaqMan allelic discrimination Assay	2
Sequencing	2*
Abbott Laboratories	1
Innogenetics Inno-LIPA	1
In-house PCR/Restriction fragment length polymorphism analysis	1
Allele-specific oligonucleotide PCR	1
In-house PCR with high resolution melt analysis	1*
Amplification/heteroduplex/restriction analysis	1
In-house single nucleotide polymorphism assay	1
Home-brew assay	1
In-house allele-specific hybridization	1
Matrix Assisted Laser Desorption /Ionization- Time Of Flight (MALDI-TOF) mass spectrometry	1
PCR with Real Time Probe and Heteroduplex Analysis (F508del only)	1*

*Assays used in addition to another method listed.

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SPECIMEN CERTIFICATION

Specimen	Allele 1 (Colloquial name)	Allele 2 (Colloquial name)	Allele 1 (Standard name)	Allele 2 (Standard name)	Expected Clinical Assessment
49C1	3120+1G→A	L467P*	c.2988+1G>A	p.L467P	2 or 3
49C2	Wild type	Wild type	Wild type	Wild type	1
49C3	621+1G→T	R1162X	c.489+1G>T	p.Arg1162X	2
49C4	F508del	F508del	p.F508del	p.F508del	2
49C5	Wild type	Wild type	Wild type	Wild type	1

1 = screen negative (normal) 2 = likely cystic fibrosis positive 3 = likely cystic fibrosis carrier

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

***Specimen 49C1 contains an allele that is not on most mutation panels and would only be found with sequencing. Additionally, it is not clear if it is a sequence polymorphism that has no effect or a disease-causing mutation. Therefore, clinical assessments of likely cystic fibrosis carrier or of likely cystic fibrosis positive were accepted as correct.**

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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