Newborn Screening Quality Assurance Program

Cystic Fibrosis DNA Variant Detection Proficiency Testing Program (CFDNAPT)

In co-sponsorship with Association of Public Health Laboratories (APHL)
Provided by the Newborn Screening and Molecular Biology Branch
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Report Authorization

This report has been reviewed and authorized by Dr. Suzanne Cordovado, Laboratory Chief, Molecular Quality Improvement Program.

Confidentiality Statement

NSQAP participant information and evaluations are strictly confidential and shared only with individual participants, unless written authorization for release is received.

Introduction

This report summarizes all results submitted within the data-reporting period for the Quarter 4, 2019 program for cystic fibrosis (CF) variant 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 primary and secondary screening methods, the DNA extraction methods used by participants, the summary of reported genotypes, and the overall summary of reported clinical assessments. An evaluation of submitted data is attached to individual laboratory reports.

Certification of PT Specimens

The Quarter 4 panel consisted of five dried blood spot (DBS) specimens (419C1, 419C2, 419C3, 419C4, and 419C5) prepared from CF patients, carriers, or unaffected individuals. All variants are characterized at CDC using Sanger sequencing and variants 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 assayed using Luminex Molecular Diagnostics xTAG CF 60 v2 to verify robust performance.

Table 1. Specimen Certification

Specimen	Allele 1	Allele 2	Genotype[§]	Clinical Assessment
419C1	F508del (c.1521_1523delCTT)	No variants detected	F508del (c.1521_1523delCTT)/+	2 (Screen Positive-1 or 2 variants)
419C2	F508del (c.1521_1523delCTT)	3905insT (c.3773dupT)	F508del (c.1521_1523delCTT)/ 3905insT (c.3773dupT)	2 (Screen Positive-1 or 2 variants)
419C3	F508del (c.1521_1523delCTT)	A455E (c.1364C>A)	F508del (c.1521_1523delCTT)/ A455E (c.1364C>A)	2 (Screen Positive-1 or 2 variants)
419C4	No variants detected	No variants detected	+/+	1 (Screen Negative-Normal)
419C5	F311del (c.933_935delCTT)	No variants detected	F311del (c.933_935delCTT)/ +	2 (Screen Positive-1 or 2 variants)

[§] The + in the genotype indicates there are no variants detected in the *CFTR* gene on one or both chromosomes.

Distribution of PT Specimens

On September 24, 2019, NSQAP distributed a panel of five unknown DBS specimens to 35 laboratories in the United States and 41 laboratories in other countries to detect variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

Participant Results

Data was received from 68 participants by the data reporting deadline. Participants tested specimens by the analytical schemes they routinely use. Reported data included method(s), DNA extraction, variant 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 variant panel or regions sequenced for the submission to be accepted.

Reported Method Data

Methods varied widely with regard to the panel of variants detected, the algorithm used for testing, and the DNA extraction methods used. Tables 2 – 4 provide the primary and secondary methods used for analysis and the DNA extraction methods reported by participants.

Table 2. Reported Primary Methods

Primary Method	# of Labs
CF1 GenMark Cystic Fibrosis Genotyping	3
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	12
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	11
CF6 Luminex Molecular Diagnostics xTAG CF 71 v2	1
CF7 Luminex Platform and Laboratory Developed Test	1
CF8 Elucigene Diagnostics CF4v2	1
CF10 Elucigene Diagnostics CF30v2	2
CF11 Elucigene Diagnostics CF-EU2v1	5
CF15 Inno–LiPA Strips 17+19	2
CF16 Sequenom HerediT CF assay	1
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	4
CF18 ViennaLab Diagnostics GmbH CF StripAssay, GER	4
CF19 ViennaLab Diagnostics GmbH CF StripAssay, 4-410	1
CF20 Allele-specific Oligonucleotide PCR	2
CF21 High Resolution Melt Technology	1
CF22 Real-time PCR Allelic Discrimination Assay (ie TaqMan)	2
CF29 Next Gen Sequencing - Illumina MiSeqDx 139 Variant Assay	3
CF32 All other gene sequencing protocols including Sanger and Next Gen	7
CF34 Devyser CFTR Core	1
CF35 Agena Bioscience iPLEX pro CFTR panel	1
CF99 Other	1

Table 3. Reported Secondary Methods

Secondary Method	# of Labs
CF1 GenMark Cystic Fibrosis Genotyping	1
CF4 Luminex Molecular Diagnostics CFTR IVD 39 v2	5
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
CF21 High Resolution Melt Technology	1
CF25 PCR/Heteroduplex Analysis/Gel Electrophoresis	2
CF31 Next Gen Sequencing - Ion AmpliSeq CFTR Community Panel	1
CF32 All other gene sequencing protocols including Sanger and Next Gen	5
CF35 Agena Bioscience iPLEX pro CFTR panel	1
CF99 Other	3

Table 4. Reported DNA Extraction Methods

Extraction Method	# of Labs
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	21
X4 Sigma Aldrich Extract-N-Amp	3
X5 in-house alkaline lysis prep	7
X6 in-house boiling prep	2
X7 in-house lysis boiling prep	3
X8 ViennaLab GenXtract	3
X9 Perkin Elmer/ Chemagen Chemagic kit	1
X19 Other	17

Allele Assessment Data

Tables 5a – 5e show the genotypes identified by the participants and the genotype errors for each specimen.

Table 5a. **Specimen 419C1**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/+	65	0
F508del (c.1521_1523delCTT)/ No allele reported	1	0

Table 5b. **Specimen 419C2**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/ 3905insT (c.3773dupT)	54	0
F508del (c.1521_1523delCTT)/+	12	1

Table 5c. **Specimen 419C3**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/ A455E (c.1364C>A)	54	0
F508del (c.1521_1523delCTT)/+	11	0
F508C (c.1523T>G)/ A455E (c.1364C>A)	1	1

Table 5d. **Specimen 419C4**

Genotype Identified	Number of labs	Number of Genotype Errors
+/+	66	0

Table 5e. **Specimen 419C5**

Genotype Identified	Number of labs	Number of Genotype Errors
F311del (c.933_935delCTT)/+	10	1
+/+	54	0
F311del (c.933_935delCTT)/ No allele reported	1	0
No alleles reported	1	0

Clinical Assessment Data

Since all specimens were evaluated based on participants' specific method(s), variant panel, and algorithm, the clinical assessments may vary between laboratories while still being correct. Table 6 provides a summary of participants' clinical assessments for each specimen.

Table 6. Clinical Assessments Reported for each Specimen

Clinical Assessment	419C1	419C2	419C3	419C4	419C5
1 (Screen Negative- Normal)	0	0	0	65	54
2 (Screen Positive – 1 or 2 variants)	65	65	65	0	10
No Clinical Assessments Reported	1	1	1	1	2
Incorrect Clinical Assessment(s)	0	0	0	0	2

Evaluations

Evaluations are based on the allele identification and clinical assessment for each specimen. A "Misclassification" is assigned if either of the alleles and/or clinical assessment reported is incorrect according to the laboratoy's panel and algorithm. Submissions were not evaluated if no data was reported for the quarter, an incorrect form was used, or if alterations were made to entries on the form. Since participants are evaluated according to their screening method(s), variant panel, and screening algorithm, the identified alleles and clinical assessments may vary from laboratory to laboratory while still being correct.

NSQAP received data from 68 participants and processed data from 66 participants. Two laboratories reported invalid entries and were not evaluated. Eight laboratories did not report data for Quarter 4 of 2019.

Summary of Overall Evaluations for each Specimen

Specimen 419C1

- 65 participants reported a clinical assessment of screen positive
- 1 participant did not report a clinical assesment
- 1 participant did not report an allele

Specimen 419C2

- 65 participants reported a clinical assessment of screen positive
- 1 participant did not report a clinical assessment
- 1 participant did not detect an allele present in their panel

Specimen 419C3

- 65 participants reported a clinical assessment of screen positive
- 1 participant did not report a clinical assessment
- 1 participant did not detect an allele present in their panel and reported an incorrect allele

Specimen 419C4

- 65 participants reported a clinical assessment of screen negative
- 1 participant did not report a clinical assessment

Specimen 419C5

- 54 participants reported a clinical assessment of screen negative
- 10 participants reported a clinical assessment of screen positive
- 2 participants did not report a clinical assessment (1 of which reported amplification failure)
- 1 participant detected an allele not present on their reported panel, resulting in an incorrect clinical assessment
- 1 participant correctly detected the alleles present, but reported an incorrect clinical assessment

Future Shipments

The Newborn Screening Quality Assurance Program will ship Quarter 1 PT specimens for the CFDNAPT on January 14, 2020.

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The content of this report may also be located on our website at:
https://www.cdc.gov/labstandards/nsqap_reports.html

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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