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, 2018 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 and the DNA extraction methods used by participants, the overall summary of reported genotypes, and the overall summary of clinical assessments reported. 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 (418C1, 418C2, 418C3, 418C4, and 418C5) 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
418C1	F508del (c.1521_1523delCTT)	F508del (c.1521_1523delCTT)	F508del (c.1521_1523delCTT)/ F508del (c.1521_1523delCTT)	2 (Screen Positive-1 or 2 variants)
418C2	No variants detected	No variants detected	+/+	1 (Screen Negative-Normal)
418C3	F508del (c.1521_1523delCTT)	E60X (c.178G>T)	F508del (c.1521_1523delCTT)/ E60X (c.178G>T)	2 (Screen Positive-1 or 2 variants)
418C4	G542X (c.1624G>T)	3849+10kbC>T* (c.3717+12191C>T)	G542X (c.1624G>T)/ 3849+10kbC>T (c.3717+12191C>T)	2 (Screen Positive-1 or 2 variants)
418C5	2183AA>G (c.2051_2052delAAinsG)	No variants detected	2183AA>G (c.2051_2052delAAinsG)/+	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.

* The 3849+10kbC>T (c.3717+12191C>T) variant is also described as (c.3818-2477C>T).

Distribution of PT Specimens

On September 25, 2018, 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 70 participants by the data reporting deadline. Participants tested specimens by the analytical schemes they routinely use. Reported data included method(s), 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	15
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	3
CF11 Elucigene Diagnostics CF-EU2v1	5
CF15 Inno–LiPA Strips 17+19	2
CF16 Sequenom HerediT CF assay	2
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	3
CF18 ViennaLab Diagnostics GmbH CF StripAssay, GER	3
CF19 ViennaLab Diagnostics GmbH CF StripAssay, 4-410	1
CF20 Allele-specific Oligonucleotide PCR	1
CF21 High Resolution Melt Technology	1
CF22 Real-time PCR Allelic Discrimination Assay (ie TaqMan)	2
CF25 PCR/Heteroduplex Analysis/Gel Electrophoresis	2
CF27 Amplification and Restriction Fragment Length Polymorphism Analysis	1
CF29 Next Gen Sequencing - Illumina MiSeqDx 139 Variant Assay	3
CF30 Next Gen Sequencing - Multiplicom Molecular Diagnostics CFTR MASTR v2	1
CF32 All other gene sequencing protocols including Sanger and Next Gen	3
CF34 Devyser CFTR Core	1
CF35 Agena Bioscience iPLEX pro CFTR panel	1
CF37 Swift Biosciences Accel Amplicon CFTR Panel	1
CF99 Other	2

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	2
CF15 Inno–LiPA Strips 17+19	3
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	2
CF18 ViennaLab Diagnostics GmbH CF StripAssay, GER	1
CF21 High Resolution Melt Technology	1
CF26 Capillary Electrophoresis	2
CF31 Next Gen Sequencing - Ion AmpliSeq CFTR Community Panel	1
CF32 All other gene sequencing protocols including Sanger and Next Gen	9
CF35 Agena Bioscience iPLEX pro CFTR panel	1
CF99 Other	5
No secondary method reported	35

Table 4. Reported DNA Extraction Methods

Extraction Method	# of Labs
X1 Qiagen QIAamp spin columns (manual or robotic)	8
X2 Qiagen magnetic bead kit (EZ1 or BioSprint 96)	2
X3 Qiagen Generation DNA Purification & DNA Elution Solutions	23
X4 Sigma Aldrich Extract-N-Amp	3
X5 in-house alkaline lysis prep	9
X6 in-house boiling prep	3
X7 in-house lysis boiling prep	2
X8 ViennaLab GenXtract	2
X9 Perkin Elmer/ Chemagen Chemagic kit	1
X19 Other	17

Allele Assessment Data

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

Table 5a. **Specimen 418C1**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/ F508del (c.1521_1523delCTT)	68	0
F508del (c.1521_1523delCTT)/+	1	1
F508del (c.1521_1523delCTT)/ F508C (c.1523T>G)	1	1

Table 5b. **Specimen 418C2**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/+	1	1
No Variants Detected	67	0
No Variants Reported	2	0

Table 5c. **Specimen 418C3**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/ E60X (c.178G>T)	38	0
F508del (c.1521_1523delCTT)/+	30	0
F508C (c.1523T>G)/ E60X (c.178G>T)	1	1
F508del (c.1521_1523delCTT)/ No Variant Reported	1	0

Table 5d. **Specimen 418C4**

Genotype Identified	Number of labs	Number of Genotype Errors
G542X (c.1624G>T)/ 3849+10kbC>T (c.3717+12191C>T)	59	0
G542X (c.1624G>T)/+	7	3
No Variants Detected	3	0
No Variants Reported	1	0

Table 5e. **Specimen 418C5**

Genotype Identified	Number of labs	Number of Genotype Errors
2183AA>G (c.2051_2052delAAinsG)/+	50	0
2184delA (c.2052delA)/+	6*	2
2183AA>G (c.2051_2052delAAinsG)/ No Variant Reported	1	0
No Variants Detected	12	0
No Variants Reported	1	0

* For one of the reported screening methods, it is documented in the Manufacture's instructions that cross reactivity is known to occur when a specimen contains a 2183AA>G (c.2051_2052delAAinsG) variant, resulting in a 2184delA (c.2052delA) call, hence in this situation, a 2184delA call is not considered a genotyping error.

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 the participants' clinical assessments for each specimen.

Table 6. Clinical Assessments Reported for each Specimen

Clinical Assessment	418C1	418C2	418C3	418C4	418C5
Screen Negative	0	68	0	3	13
Screen Positive (1 or 2 Variants Detected)	69	1	69	66	56
Clinical Assessment Not Reported	1	1	1	1	1
Incorrect Clinical Assessment(s)	0	1	0	0	1

Evaluations

Evaluations are based on the allele assessment and clinical assessment of each specimen where the clinical assessment counts for 10% of the assessment and each identified allele counts for 5% of the assessment. Since participants are graded 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 and processed data from 70 participants. One participant did not report clinical assessments for all specimens. Six laboratories did not report data for Quarter 4 of 2018.

Summary of Overall Evaluations for each Specimen

Specimen 418C1

- 69 participants reported a clinical assessment of screen positive
- 1 participant did not detect an allele present in their variant panel, but it did not result in an incorrect clinical assessment
- 1 participant detected an incorrect allele, but it did not result in an incorrect clinical assessment

Specimen 418C2

- 68 participants reported a clinical assessment of screen negative
- 1 participants reported a clinical assessment of screen positive
- 2 participants did not report alleles for this specimen
- 1 participant detected an incorrect allele that resulted in an incorrect clinical assessment

Specimen 418C3

- 69 participants reported a clinical assessment of screen positive
- 1 participant detected an incorrect allele, but it did not result in an incorrect clinical assessment
- 1 participant did not report any alleles for this specimen

Specimen 418C4

- 3 participants reported a clinical assessment of screen negative
- 66 participants reported a clinical assessment of screen positive
- 3 participants did not detect an allele present in their variant panel, but it did not result in an incorrect clinical assessment

Specimen 418C5

- 13 participants reported a clinical assessment of screen negative
- 56 participants reported a clinical assessment of screen positive
- 2 participants did not report one or both alleles for this specimen
- 1 participant detected the correct alleles, however reported an incorrect clinical assessment

Future Shipments

The Newborn Screening Quality Assurance Program will ship Quarter 1 PT specimens for the CFDNAPT on January 15, 2019.

Direct Inquiries

If you have any comments or questions about CFDNAPT, contact Dr. Suzanne Cordovado at 770-488-4048 or by email at SCordovado@cdc.gov

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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

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