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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Quarterly Report
Volume 14, No.1
Issued: March 31, 2020

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 1, 2020 proficiency testing 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 summary of reported genotypes, and the overall summary of reported clinical assessments. An evaluation of submitted individual laboratory data is attached.

Certification of PT Specimens

The Quarter 1 panel consisted of five dried blood spot (DBS) specimens (2011101, 2011102, 2011103, 2011104, and 2011105) prepared from CF patients, carriers, or unaffected individuals. All variants are characterized at CDC using Sanger sequencing and then confirmed in DBS specimens using next generation sequencing technologies and/or a multiplex genotyping assay. Prior to this distribution, DNA was extracted from DBS samples with Qiagen Generation DNA Purification and DNA Elution Solutions and an in-house boiling prep method and was assayed using Luminex Molecular Diagnostics xTAG CF 60 v2 to verify continued robust performance.

Table 1. Specimen Certification

Specimen	Allele 1	Allele 2	Genotype[§]	Clinical Assessment
2011101	F508del (c.1521_1523delCTT)	No variants detected	F508del (c.1521_1523delCTT)/+	2 (Screen Positive-1 or 2 variants)
2011102	2183AA>G (c.2051_2053delAAinsG)	3849+10kbC>T (c.3717+12191C>T)	2183AA>G (c.2051_2053delAAinsG)/ 3849+10kbC>T (c.3717+12191C>T)	2 (Screen Positive-1 or 2 variants)
2011103	No variants detected	No variants detected	+/+	1 (Screen Negative-Normal)
2011104	F508del (c.1521_1523delCTT)	1717-1G>A (c.1585G>A)	F508del (c.1521_1523delCTT)/ 1717-1G>A (c.1585G>A)	2 (Screen Positive-1 or 2 variants)
2011105	N1303K (c.3909C>G)	D1152H (c.3454G>C)	N1303K (c.3909C>G)/ D1152H (c.3454G>C)	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 January 14, 2020, NSQAP distributed a panel of five unknown DBS specimens to 35 laboratories in the United States and 42 laboratories in other countries to detect variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene.

Participant Results

Data was received from 73 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	13
CF5 Luminex Molecular Diagnostics xTAG CF 60 v2	11
CF6 Luminex Molecular Diagnostics xTAG CF 71 v2	2
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	1
CF17 Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)	5
CF18 ViennaLab Diagnostics GmbH CF StripAssay, GER	4
CF19 ViennaLab Diagnostics GmbH CF StripAssay, 4-410	2
CF20 Allele-specific Oligonucleotide PCR	2
CF21 High Resolution Melt Technology	1
CF22 Real-time PCR Allelic Discrimination Assay (ie TaqMan)	2
CF23 In-house Amplification Refractory Mutation System	1
CF29 Next Gen Sequencing - Illumina MiSeqDx 139 Variant Assay	4
CF30 Next Gen Sequencing – Multiplicom Molecular Diagnostics CFTR	2
CF32 All other gene sequencing protocols including Sanger and Next Gen	6
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	2
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
CF26 Capillary Electrophoresis	1
CF31 Next Gen Sequencing - Ion AmpliSeq CFTR Community Panel	1
CF32 All other gene sequencing protocols including Sanger and Next Gen	7
CF35 Agena Bioscience iPLEX pro CFTR panel	1
CF99 Other	4

Table 4. Reported DNA Extraction Methods

Extraction Method	# of Labs
X1 Qiagen QIAamp spin columns (manual or robotic)	9
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	2
X7 in-house lysis boiling prep	1
X8 ViennaLab GenXtract	3
X9 Perkin Elmer/ Chemagen Chemagic kit	1
X19 Other	20

Allele Assessment Data

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

Table 5a. **Specimen 2011101**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/+	73	0

Table 5b. **Specimen 2011102**

Genotype Identified	Number of labs	Number of Genotype Errors
2183AA>G (c.2051_2053delAAinsG)/ 3849+10kbC>T (c.3717+12191C>T)	55	0
2184delA (c.2052delA)/ 3849+10kbC>T (c.3717+12191C>T)	5	1
3849+10kbC>T (c.3717+12191C>T)/ +	5	0
2183AA>G (c.2051_2053delAAinsG)/+	1	0
+/+	7	0

Table 5c. **Specimen 2011103**

Genotype Identified	Number of labs	Number of Genotype Errors
+/+	73	0

Table 5d. **Specimen 2011104**

Genotype Identified	Number of labs	Number of Genotype Errors
F508del (c.1521_1523delCTT)/ 1717-1G>A (c.1585G>A)	67	0
F508del (c.1521_1523delCTT)/+	6	0

Table 5e. **Specimen 2011105**

Genotype Identified	Number of labs	Number of Genotype Errors
N1303K (c.3909C>G)/ D1152H (c.3454G>C)	31	0
N1303K (c.3909C>G)/ +	33	1
D1152H (c.3454G>C)/ +	1	1
F508del (c.1521_1523delCTT)/ N1303K (c.3909C>G)	1	1
+/+	7	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	2011101	2011102	2011103	2011104	2011105
1 (Screen Negative- Normal)	0	7	73	0	7
2 (Screen Positive – 1 or 2 variants)	73	66	0	73	66
Incorrect Clinical Assessment(s)	0	0	0	0	0

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 laboratory'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 and processed data from 73 participants. Four laboratories did not report data for Quarter 1 of 2020.

Summary of Overall Evaluations for each Specimen

Specimen 2011101

- 73 participants reported a clinical assessment of screen positive

Specimen 2011102

- 7 participants reported a clinical assessment of screen negative
- 66 participants reported a clinical assessment of screen positive
- No incorrect clinical assessments were reported, however:
    - 1 participant did not report a correct allele, which is detectable using their reported screening methods

Specimen 2011103

- 73 participants reported a clinical assessment of screen negative

Specimen 2011104

- 73 participants reported a clinical assessment of screen positive

Specimen 2011105

- 7 participants reported a clinical assessment of screen negative
- 66 participants reported a clinical assessment of screen positive
- No incorrect clinical assessments were reported, however:
    - 1 participant did not detect an allele on their variant panel
    - 1 participant detected an incorrect allele
    - 1 participant did not identify an allele which is detectable using their reported screening method

Future Shipments

The Newborn Screening Quality Assurance Program will ship Quarter 3 PT specimens for the CFDNAPT on June 23, 2020.

Acknowledgements

We would like to thank Philip Farrell, M.D., Ph.D. (University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin), Martin Kharrazi, Ph.D. (California Department of Public Health, Richmond, California), Charlene Sacramento (Sequoia Foundation, La Jolla, California) and all the CF Care Clinics 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.

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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