Proficiency Testing Assay Instructions for Cystic Fibrosis Variant Detection (CFDNAPT)

CAUTION

These specimens are made from normal donors and donors that have *CFTR* variants of interest and have not been tested for hepatitis B, HIV, and hepatitis C. Because no test method offers complete assurance that these or other infectious agents are absent, treat all specimens as potentially infectious and follow universal precautions. For more information on bloodborne pathogens visit https://www.cdc.gov/niosh/topics/bbp/

SPECIMEN QUALITY STATEMENT

NSQAP strives to create specimens that mimic newborn dried blood spots. Prepared specimens have been certified and may depart from established visual criteria for assessing specimen quality. These specimens are fit for the purposes of proficiency testing.

CONFIDENTIALITY STATEMENT

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

GENERAL INFORMATION

This program specifically targets DNA testing for variants in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. This is a separate program from the routine IRT PT program. For clinical assessment purposes, assume that the IRT is above your program's regular or ultra-high cutoff so that all samples should be tested for CFTR variants.

- All PT samples are evaluated for all participants based on each participant's variant panel and scoring algorithm. When the reported alleles or clinical assessment disagrees with what is expected or if alleles or clinical assessment fields are left blank, the result will be categorized as "unacceptable".
- **A drop down menu is provided for how and when a secondary method is used. If the user does not specify this information, it will be assumed that both methods are used in testing and all variants in both panels will be used for grading purposes.
- Note, you must use the current version of the CFDNAPT Data Reporting form—some method codes have changed so make sure the code you select is correct. DO NOT make any changes to the form format (ie do not insert or delete any cells etc.) and do not paste information into cells that are formatted with a drop-down list.
- Participants enrolled in the CFDNAPT Program will be moved to an inactive status if data is not reported for 3 consecutive quarters.

ASSAYING AND REPORTING INSTRUCTIONS

1. Inspect all proficiency testing (PT) specimens upon receipt. If a panel is incomplete or contains unlabeled specimens, request a new panel within 48 hours. Send the following information to NSQAPDMT@cdc.gov: laboratory code number, PT Panel Type, Specimen Number(s), and reason for requesting new panel.
2. Refrigerate the enclosed specimens at 4°C upon receipt if storage is necessary.
3. Handle these specimens as routine specimens. Assay them as part of your normal daily workload.

   Participating laboratories must generate and submit their own results and must not share NSQAP PT test results or specimens with any other laboratory under ANY circumstance, even if the laboratory normally sends specimens to referral laboratories for routine or confirmatory testing. Participants found to have falsified or shared results will be barred from participation in the NSQAP PT program.
4. Punch all dried blood disks for analysis from within the blood spots on the specimen cards.
5. Download the CFDNAPT Data Reporting form from our website at: https://www.cdc.gov/labstandards/nsqap_resources.html. **Any data submission form which is NOT current OR has been altered will NOT be accepted.**

6. After the analysis is complete, record (1) method codes (primary and secondary/confirmatory), (2) variant panel if not a commercial panel or deviations from a commercial panel, (3) regions sequenced if you are using a gene sequencing method, (4) when and how you use your secondary/confirmatory method, and (5) DNA extraction method on the data report form. Note: if you use legacy nomenclature when describing regions sequenced (ie. exons and introns), you must specify that you are using legacy nomenclature; otherwise, it will be assumed that you are using HGVS nomenclature.
7. Complete each assessment based on your assay results, and enter both the clinical assessment and genotype results into the designated area of the report form. Every enclosed specimen should be treated as a full-term (>2500g) baby 24 hours of age who is on no medication, has not had a blood transfusion, and has had sufficient intake of a protein and lactose-based diet for detection of any metabolic disorder.
8. There is a limited amount of specimens available for this program. If data are not reported, provide an explanation of how the specimens were used and why no data were reported in the Comments section of the data report form. If no data or explanations are given, shipments will be discontinued.
9. Attach the file to an email and send to NSQAPDMT@cdc.gov. **Include your laboratory code number in the subject line of your email.**

Late data will not be accepted for any reason. If data are not reported once within three events, your laboratory will be inactivated for this analyte program.

To view dates for future shipments, see the NSQAP Shipping Schedule at: https://www.cdc.gov/labstandards/nsqap_resources.html. For questions, send an email to **NSQAPDMT@cdc.gov** and include your **laboratory code** in the email subject line.

CF METHOD CODE LIST

CF1 - GenMark Cystic Fibrosis Genotyping
CF3 - Luminex Molecular Diagnostics xTAG CF - ACMG only
CF4 - Luminex Molecular Diagnostics CFTR IVD 39 v2
CF5 - Luminex Molecular Diagnostics xTAG CF 60 v2
CF6 - Luminex Molecular Diagnostics xTAG CF 71 v2
CF7 - Luminex Platform and Laboratory Developed Test
CF8 - Elucigene Diagnostics CF4v2
CF9 - Elucigene Diagnostics CF29v2
CF10 - Elucigene Diagnostics CF30v2
CF11 - Elucigene Diagnostics CF-EU2v1
CF12 - Abbott Molecular CF Genotyping Assay v3
CF13 - Inno–LiPA Strip 17
CF14 - Inno–LiPA Strip 19
CF15 - Inno–LiPA Strips 17+19
CF16 - Sequenom HerediT CF assay
CF17 - Sequenom assays other than HerediT CF (MALDI-TOF Mass Spectrometry)
CF18 - ViennaLab Diagnostics GmbH CF StripAssay, GER
CF19 - ViennaLab Diagnostics GmbH CF StripAssay (4-410)
CF20 - Allele-specific Oligonucleotide PCR
CF21 - High Resolution Melt Technology
CF22 - Real-time PCR Allelic Discrimination Assay (i.e. TaqMan)
CF23 - In-house Amplification Refractory Mutation System
CF24 - In-house single nucleotide primer extension assay (SNuPe)
CF25 - PCR/Heteroduplex Analysis/Gel Electrophoresis
CF26 - Capillary Electrophoresis
CF27 - Amplification and Restriction Fragment Length Polymorphism Analysis (PCR-RFLP)
CF28 - Amplification and Polyacrylamide Gel Electrophoresis (PCR-PAGE)
CF29 - Next Gen Sequencing - Illumina MiSeqDx 139 Variant Assay
CF30 - Next Gen Sequencing - Multiplicom Molecular Diagnostics CFTR MASTR v2
CF31 - Next Gen Sequencing - Ion AmpliSeq CFTR Community Panel
CF32 - All other gene sequencing protocols including Sanger and Next Gen
CF33 - Astra Biotech CFcheck DE-31

CF34 - Devyser CFTR Core
CF19 - ViennaLab Diagnostics GmbH CF StripAssay (4-410)
CF35 - Agena Bioscience iPLEX Pro CFTR Panel (72 mutations)
CF36 - In-house Hydrolysis Probe Assay
CF37 - Swift Biosciences Accel-Amplicon CFTR Panel
CF99 - Other - Please specify

UTILIZATION OF SECONDARY/CONFIRMATORY METHODS

M1 - Secondary method run only when primary method is positive and only for confirmation (NO new variants identified)
M2 - Secondary method run only when primary method is positive and may find additional variants
M3 - Both the primary and secondary methods are used to detect variants
M4 - Other -please describe below

EXTRACTION METHODS

X1 - Qiagen QIAamp spin columns (manual or robotic)
X2 - Qiagen magnetic bead kit (EZ1 or BioSprint 96)
X3 - Qiagen Generation DNA Purification & DNA Elution Solutions (also sold as 5 Prime Easy PCR Solutions 1 & 2)
X4 - Sigma Aldrich Extract-N-Amp
X5 - in-house alkaline lysis prep
X6 - in-house boiling prep
X7 - in-house lysis boil prep
X8 - ViennaLab GenXtract
X9 - Perkin Elmer/Chemagen Chemagic kit
X10 - in-house Chelex method
X19 - Other-please describe below