Quality Assurance Program
for Cystic Fibrosis Newborn Screening

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APHL Training Course
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IRT PT versus CF DNA PT

- 153 Participants
- 1 Analyte
- 7 Methods
  - 2 kits used in US
  - 5 kits used internationally
  - All commercially available

- 63 Participants
- 1 to >71 “Analytes”
- 16 Methods
  - 3 kits + 2 LDTs used in US
  - 5 kits and 10 LDTs used internationally
  - 6 kits commercially available
CF Mutation Detection Pilot Proficiency Testing Program

- Began as a collaborative effort between CDC and 3 CF Centers
- Specimens drawn from adult or adolescent CF patients and are NOT enriched with IRT (No IRT testing done).
- Began quarterly shipments in February 2007
- Program has grown from 25 to 63 laboratories
- Repository contains all of the ACMG recommended mutations and additional mutations
Recent Modification to CF DNA PT Program

- **2007-2011 Clinical assessments**
  - No mutations detected
  - 1 mutation – presumptive carrier
  - 2 mutations – presumptive CF case

- **2012 - Clinical assessments**
  - Screen Negative (No mutations detected)
  - Screen Positive (1 or 2 mutations detected)

- **Why?**
  - In line with how U.S. NBS labs report their results
  - Reduces complexity of data analysis
Many Different Methods*

- Luminex xTAG CF 39/60 v2 (Luminex Platform)
- Hologic CF Inplex Assay (Invader)
- Innogenetics Inno-Lipa (Hybridization)
- Abbott Diagnostics Oligonucleotide Ligation Assay
- Genprobe Diagnostics Elucigene (ARMS; 4 different kits – 4, 29, 30, 50 mutations)
- MALDI-TOF mass spectrometry
- In-house (TaqMan, SNuPe, hydolysis probe, etc.)
- High Resolution Melting Temperature assay
- Amplification/gel electrophoresis
- Amplification/Heteroduplex/restriction analysis
- Sequencing

*Many international labs use 2 of the listed methods
Many Different Mutation Panels

- **United States**
  - 3 kits + CA panel = 70 mutations (25 are CA-specific)

- **Poland**
  - 16 most common mutations in Polish population

- **Argentina**
  - 19 or 32 mutations

- **Wales**
  - 8 mutations (1 specific to Welsh population)

- **France**
  - 30 mutations (kit developed specifically for their population)

- **UK**
  - 4 or 29 mutations (common to Europe)

**TOTAL = 119 mutations; only 1 (F508del) is common to all**
Most Common Issues

- **Laboratory space**
  - Pre- and post PCR space

- **Vocabulary**
  - Homozygote, heterozygote, compound heterozygote

- **Contamination**
  - Specific protocols must be followed

- **Complex Assays → Complex Troubleshooting**

- **Screening vs diagnosis**

**EXTRACTION**
Quality Control Materials
CF and Beyond
Section 10.3.9 Quality Control (2nd tier assays)

- Not practical to analyze controls for all mutations in every run,
- Permissible to include
  - a common mutation (e.g., F508del)
  - a non-template control to determine (contamination)
  - One or more of the other mutations in the panel
- Should not report the presence of mutations for which there is no external control material
- QC material preferably in DBS matrix to evaluate entire process (DNA extraction through genotype)
Laboratory-Created Molecular QC Materials
CFTR Mutation Analysis

- QA materials created from transformed cell lines
- Cell lines available for ACMG 23
- Actively working towards covering all mutations tested for in the US
- Pilot testing in U.S. and Canadian labs
- Currently, 90-93% of labs report accurate genotypes
- Based on these results, low DNA extraction efficiency is causing genotyping failures
Laboratories that could not genotype the specimen were the same for each genotype.
QC Needs of NBS Laboratories
2011 NBS & GT Symposium Poll Results

Would your laboratory find a molecular QA for Galactosemia useful?
N=96

- Yes: 53.1%
- No: 15.6%
- N/A (Do not work in a NBS lab): 31.2%
QC Needs of NBS Laboratories
2011 NBS & GT Symposium Poll Results

Would your laboratory find a molecular QA for MCAD useful?
N=85

- Yes: 42.4%
- No: 27.1%
- N/A (Do not work in a NBS lab): 30.6%
QC Needs of NBS Laboratories
2011 NBS & GT Symposium Poll Results

Would your laboratory find a molecular QA for Hemoglobinopathies useful?
N=94

- Yes: 41.5%
- No: 26.6%
- N/A (Do not work in a NBS lab): 31.9%
QC Needs of NBS Laboratories
2011 NBS & GT Symposium Poll Results

Would your laboratory find a molecular QA for Congenital Adrenal Hyperplasia useful?
N=89
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