Quality Assurance Program for Cystic Fibrosis Newborn Screening

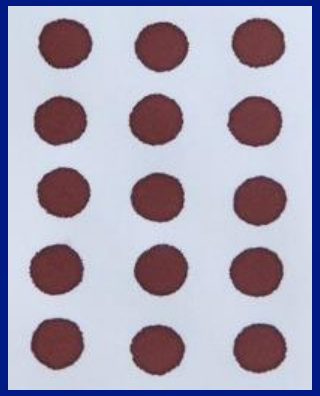
Marie C. Earley, Ph.D.
Research Microbiologist

APHL Training Course
March 9-13, 2015

National Center for Environmental Health
Division of Laboratory Sciences

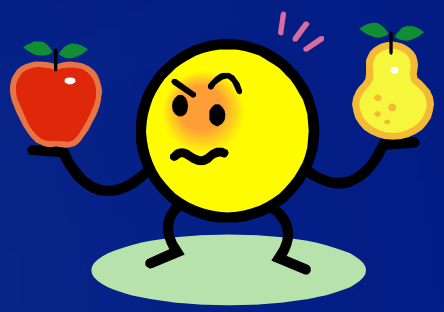
CF Mutation Detection Proficiency Testing Program

- 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 64 laboratories covering 18 countries
- Repository contains all of the ACMG recommended mutations and additional mutations



IRT PT versus CF DNA PT

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



- 64 Participants
- 1 to ≥71 “Analytes”
- 28 Methods
  - 3 kits (FDA-approved) + 4 LDTs used in US
  - 11 kits and 11 LDTs used internationally
  - Some assays are not commercially available

Many Different Methods*

- *Luminex xTAG CF 39/60/v2*
- *Hologic CF Inplex Assay 23 or 40+4 (Invader)*
- In-house
  - Amplification/gel electrophoresis
  - TaqMan assay
  - Luminex platform

- Luminex xTAG CF 71 v2
- Innogenetics (Hybridization; 19 or 36 mutations)
- Abbott Diagnostics Oligonucleotide Ligation Assay
- Elucigene (ARMS; 4, 29, 30, or 50 mutations)
- MALDI-TOF mass spectrometry
- High Resolution Melting Temperature assay
- Amplification/Heteroduplex/restriction analysis
- Sequencing

*Many international labs use 2 or more of the listed methods

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**
- **Extraction**
  - Very common analytical issue

Modifications to CF DNA PT Program

- **2013**
  - Evaluations based on genotype and clinical assessment
  - Each allele counts as 5% and the clinical assessme counts for 10% of the score

- **Why?**
  - Laboratories sometimes had the correct clinical assessment but incorrect genotype – could have analytical problem
  - With treatments based on mutation, genotype is becoming more important (e.g. Kalydeco®)

Mutation-Specific Drug Therapy

- **Kalydeco™ (ivacaftor)**
  - Effective for Class III mutations: G551D, G551S, G178R, S549N, S549R, G1244E, S1251N, S1255P, G1349D, and R117H
  - Class III mutations cause defects that affect protein function
- **Lumacaftor**
  - Nov 2014: New drug application submitted to FDA for a combination of ivacaftor and lumacaftor for patients with F508del/F508del
- **Other pharmaceuticals**
  - Several drug companies are currently testing compounds in Phase I, Phase II, and Phase III clinical trials

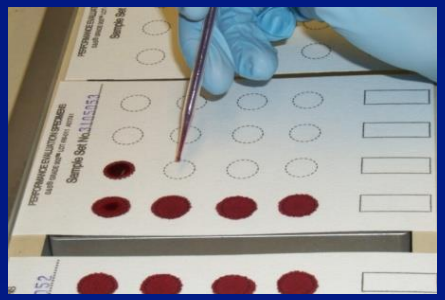
Quality Control Materials
CF and Beyond

Newborn Screening for Cystic Fibrosis; Approved Guideline
CLSI ILA35-A

- **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 detection*)

Laboratory-Created Molecular QA Materials
CFTR Mutation Analysis

- **QA materials created from transformed cell lines**
- **Continually working towards covering all mutations tested for in the US**
- **Low DNA extraction efficiency causes genotyping failures**
  - Participant feedback
  - MQIP research



DNA Yields from Common NBS DNA Extraction Methods (measured by qPCR)

	Boil (Gen)	Boil	Methanol Boil
Sample	DNA yield (ng)	DNA yield (ng)	DNA yield (ng)
Adult PT Sample 1*	44.50	6.05	4.05
Adult PT Sample 2*	122.50	32.51	8.75
Adult PT Sample 3*	289.50	54.59	19.60

* Extracted from NSQAP's Adult Cystic Fibrosis PT specimens with known high, medium and low concentrations

- **Boil Prep**
  - ~5 fold lower than Boil Prep Generation
- **Methanol Boil Prep**
  - ~13 fold lower than Boil Prep Generation

Newborn Screening Needs Compared to the Coriell Cell Repositories

Cell Line Information	Needed	Coriell
Number of ACMG recommended mutations	23	23
Number of additional mutations found in commercial assays used in U.S. newborn screening laboratories	38	18
Number of California-specific mutations	11	3
Total number of unique mutations	72	44

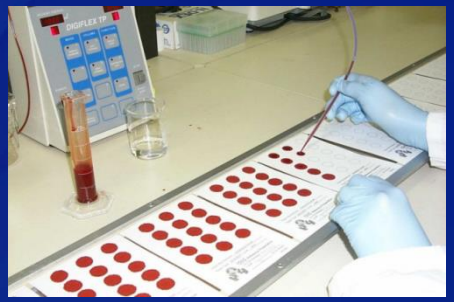
Laboratory prepared DBS for molecular assays Current Efforts

Laboratory efforts

- Test DBS created with cell lines for CF and Galactosemia
- Transform cells to immortalize
- Collect blood with rare mutations

Based on pilot testing

- Determine criteria for use as PT specimens
- Determine certification criteria for use as QC specimens



Additions to NSMBB CF Repository (DBS & cryopreserved cells)

New Additions to NSMBB CF Repository	Received
Total number of mutations received* (February 2015)	71
Number of ACMG recommended mutations replenished	19
Number of mutations in commercial assays used by U.S. newborn screening laboratories (non-ACMG)	21
Number of California-specific mutations	7

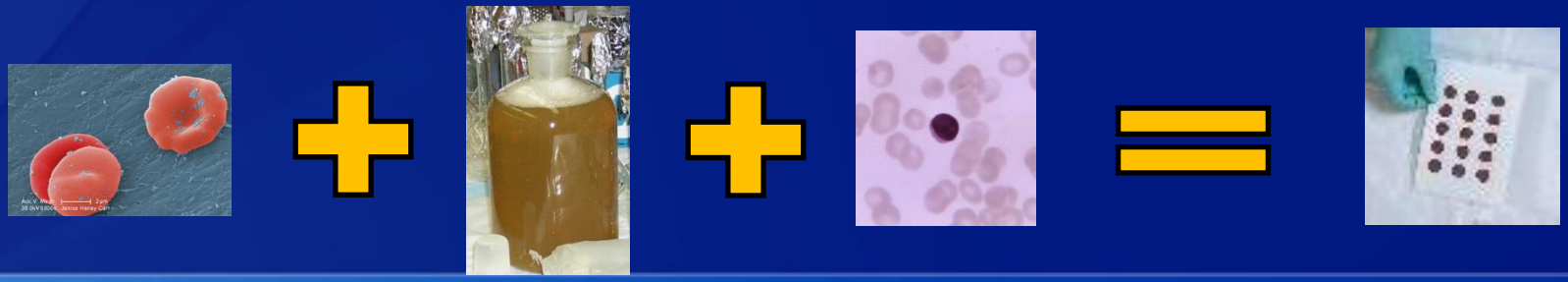
*Mutations requested and found through sequencing

Diversity of Donors – Place of Birth

Country	Number of Donors
United States	73
Mexico	14
Guatemala	5
El Salvador	3
Iran	2
India	2
Canada	2
Iceland	1
Germany	1
Japan	1
Not provided	6

Moving Forward

- **Pilot DBS for galactosemia mutations created by MQIP**
  - Good news: labs testing for 9 mutations or less
  - Bad news: all laboratory developed assays
- **DBS derived from immortalized cell lines**
- **Molecular PT program expansion**
  - Disorders on RUSP that use a molecular assay (galactosemia)
  - Exploring development of a CAH 2nd tier molecular test
  - Disorders recommended by APHL Molecular Subcommittee



NSMBB CF DBS Repository

- Proficiency testing
- Validation/Verification of methods
- Troubleshooting
- Working toward covering mutation panels for all of the methods used in the US

Take-Home Messages

- **Typical challenges in NBS labs doing molecular assays**
  - Lab space – Unidirectional work flow
  - Contamination – previous amplicons contaminate new runs
  - Vocabulary/nomenclature – may not be familiar with terms
  - Complex assays to troubleshoot – many steps or many mutations
  - DNA extraction - efficiency and purity may affect assay
- **CF PT program evaluates genotype & clinical assessments**
- **Mutation specific drug therapy now available**
  - Kalydeco
  - Another being evaluated by FDA
  - More in Phase II and Phase III trials
- **NSMBB has repository of DBS for PT, validation, etc.**
- **NSMBB is developing QC materials for CF molecular assays**

If you need *CFTR* materials, please contact:

Marie Earley	770-488-7828	mearley@cdc.gov
NSQAP email		nsqapdmt@cdc.gov

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333

Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348

Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info