Evaluation of Dried Blood Spot Quality Control Materials for Cystic Fibrosis Molecular Tests

Marie C. Earley, Ph.D.

Centers for Disease Control and Prevention, Atlanta, Georgia

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Objectives of Study

- Molecular testing in newborn screening (NBS) laboratories has become increasingly common
- Quality control QC materials in the DBS matrix for *CFTR* testing are not readily available
- NSQAP developed a method for constructing DBS QC materials for CF molecular testing
- U.S. NBS laboratories participating in NSQAP's CF Mutation Detection PT program have evaluated pilot materials

Flowchart of General Procedure

Grow cell lines (Coriell Cell Repositories)

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Mix lymphoblasts, red blood cells, and serum

↓

Adjust hematocrit and spot

↓

Confirm genotypes of DBS

↓

Send to CF Mutation Detection PT participants

↓

Evaluate performance based on reported genotype

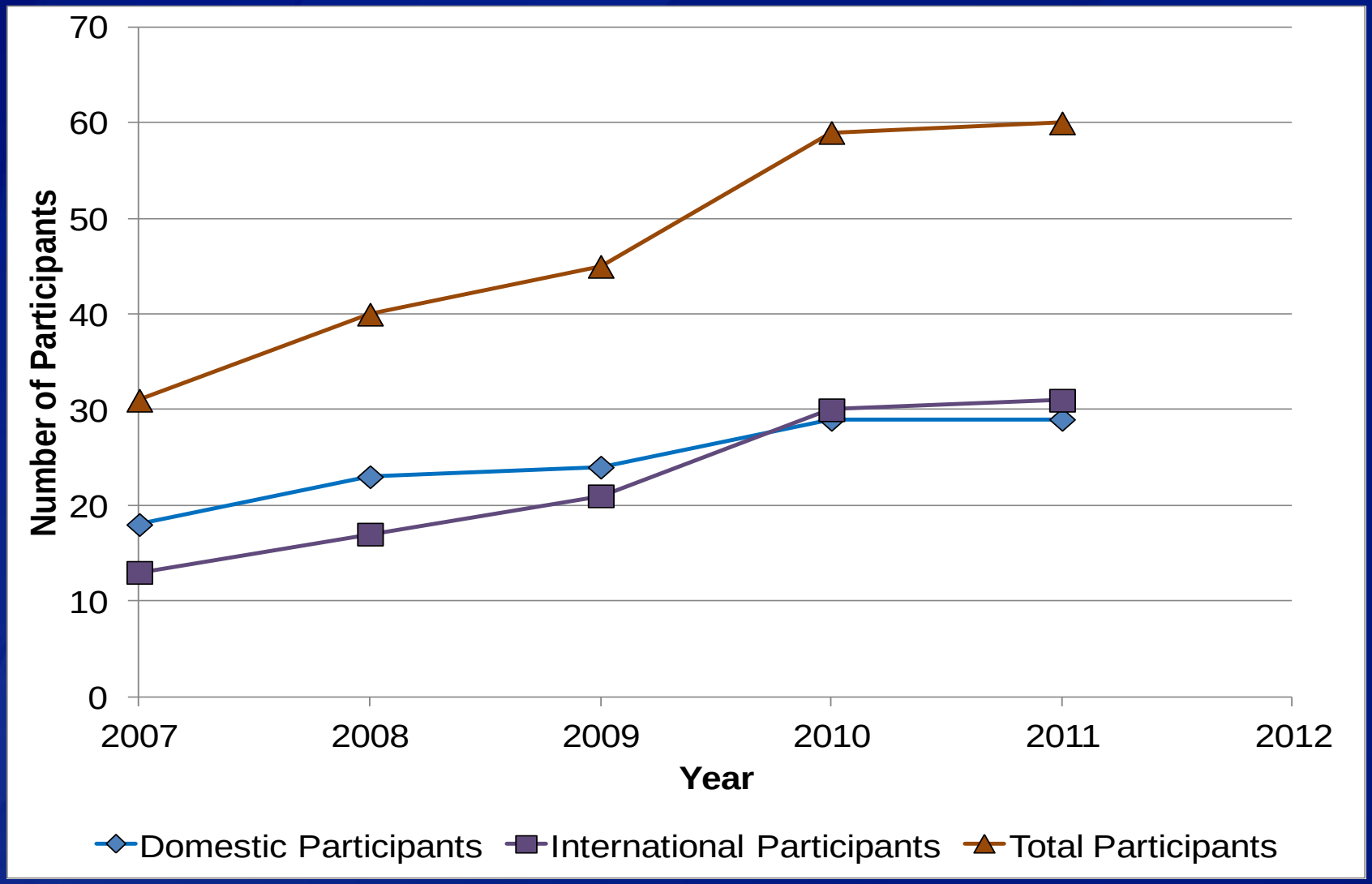
Classification of Methods and Mutations Used by Proficiency Testing Participants

	U.S.	International
# of Participating Laboratories	29	30
# of Methods Used*	5	18
kits	3	7
in-house	2	11
Total # of Mutations Covered	44	119†
Total # of Variants Covered	6	6

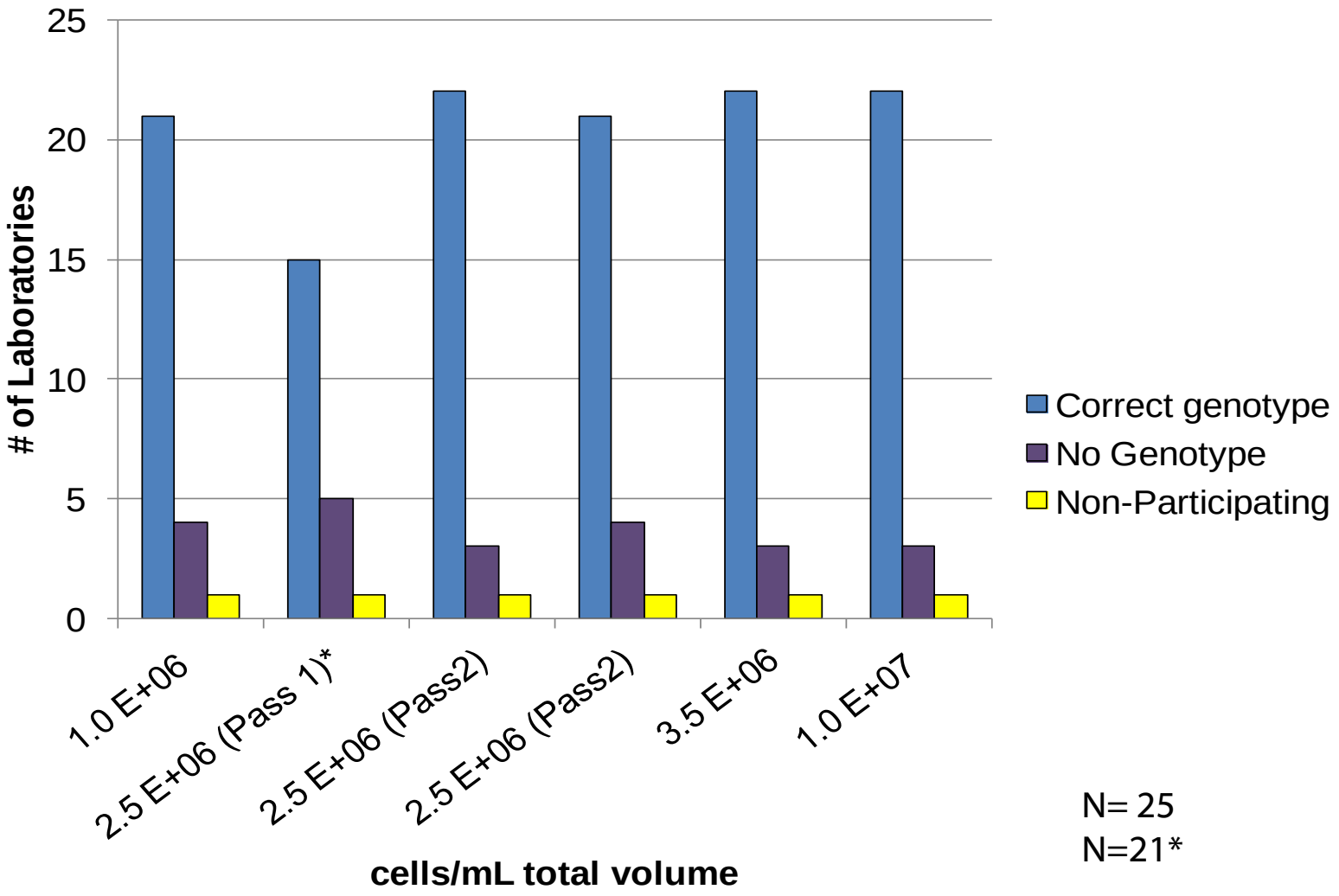
* Does not count multiple versions of same kit

† Minimum number as some methods can find many rare mutations

CF Mutation Detection Proficiency Testing Program Growth

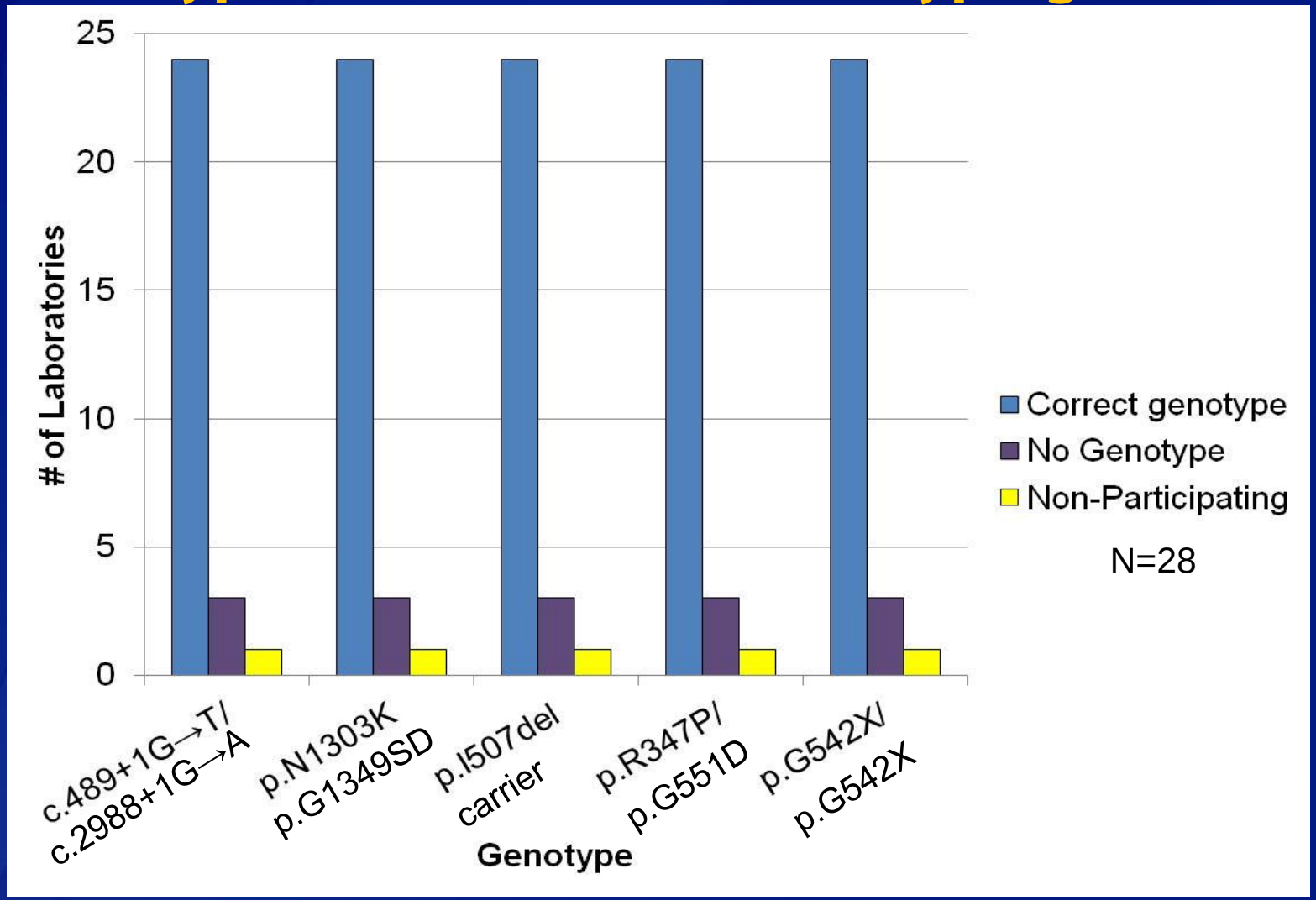


Concentration of Cells vs Genotyping Results



Based on results, all future materials were made using 3.5 X 10^6 cells/mL total volume

Genotype of Cell Lines vs Genotyping Results



Laboratories that could not genotype the specimen were the same for each genotype

Commonly Reported Issues

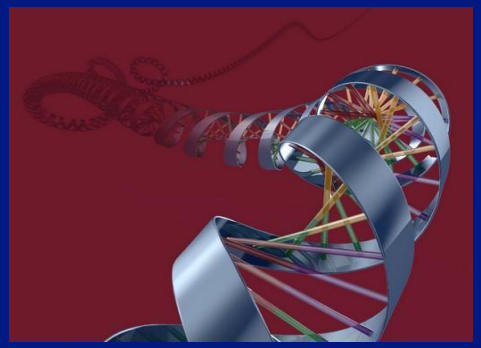
- **"Low Signal", "Equivocal" or "Sample Failure" were reported**
- **Did not always interfere with data interpretation**
- **7 laboratories could not provide a genotype in Rnd 1**
- **3 laboratories could not genotype in Rnd 2**
- **Of the remaining 4 laboratories,**
  - 1 laboratory stopped participating
  - 1 laboratory did not specify any changes to the procedure
  - 2 laboratories extracted DNA from more punches or changed the DNA extraction protocol

Conclusions

- Appropriate QC materials are important to the quality management system.
- DBS controls are needed to monitor the testing process from beginning to end
- NSQAP's pilot materials were correctly genotyped in the majority of laboratories
- An increase in cell concentration did not make a substantial difference in performance
- Difficulties in genotyping were resolved by increasing the amount of DNA extracted or the efficiency of the extraction method.

Future Activities

- Continue to monitor the performance of the materials
- Collaborate with NBS laboratories and CF Centers to add other mutations needed
- Prepare pilot materials to cover the recommended panel of 23 mutation and others
- Prepare pilot materials for other NBS disorders that use a DNA-based confirmatory test



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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
E-mail: cdcinfo@cdc.gov Web: www.cdc.gov

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