Quality Assurance Program for Cystic Fibrosis Newborn Screening

Marie C. Earley, Ph.D.
Research Microbiologist

APHL Training Course
May 8-11, 2012

National Center for Environmental Health
Newborn Screening and Molecular Biology Branch

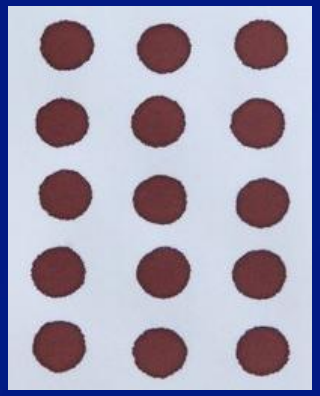
IRT PT versus CF DNA PT

- 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

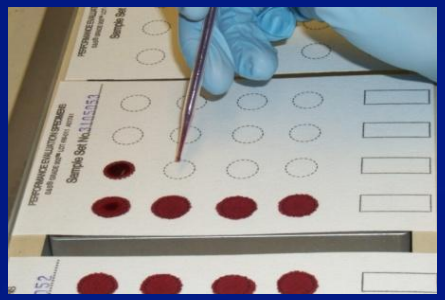
Newborn Screening for Cystic Fibrosis; Approved Guideline

CLSI ILA35-A

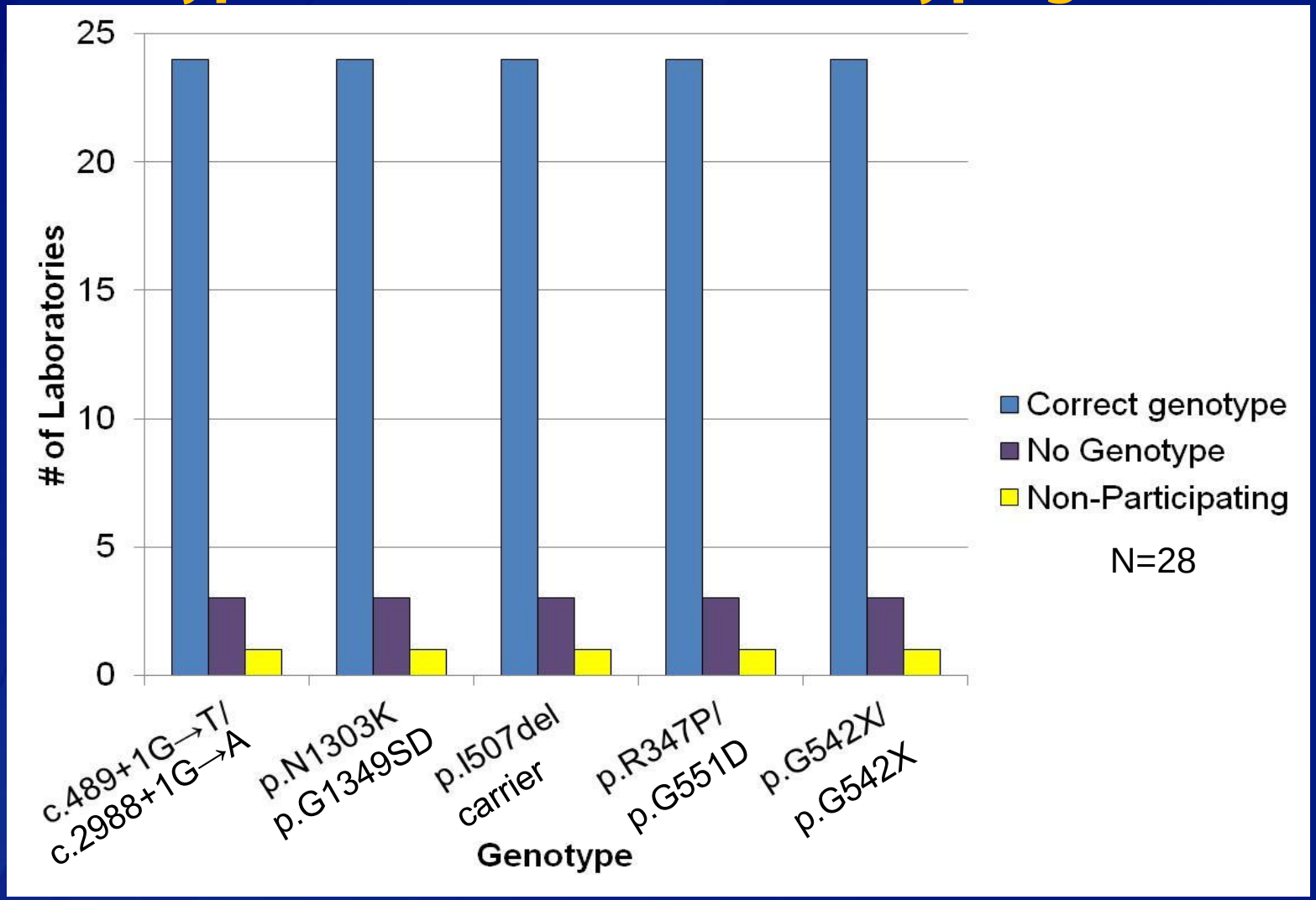
- **Section 10.3.9 Quality Control (2nd tier assays)**
  - Not practical to analyze controls for all mutations in every run,
  - Permissible to include
    - a common mutation (eg, F508del)
    - a non-template control to determine (contamination)
    - One or more of the other mutations in the panel
  - 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



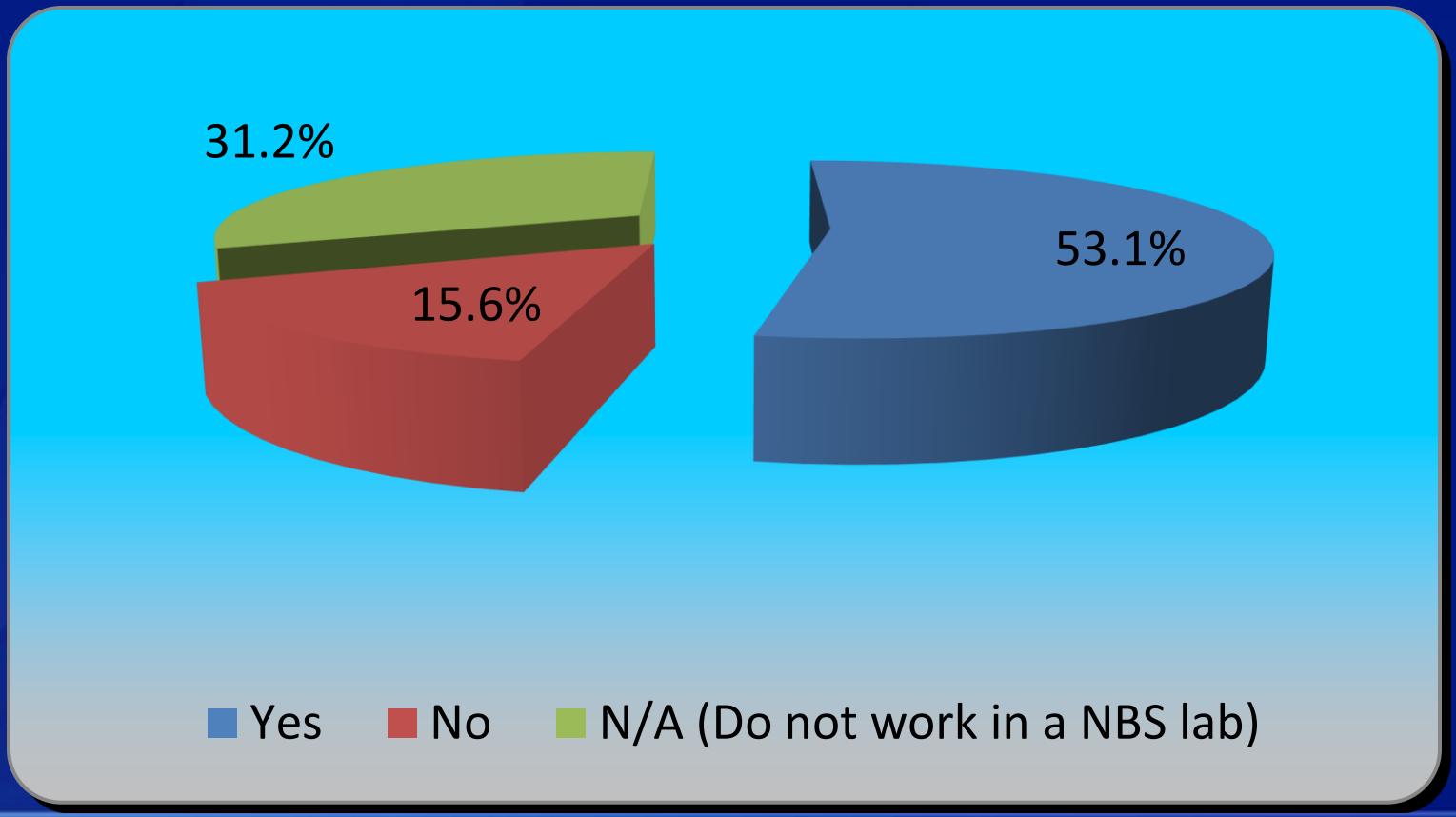
Genotype of Cell Lines vs Genotyping Results



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

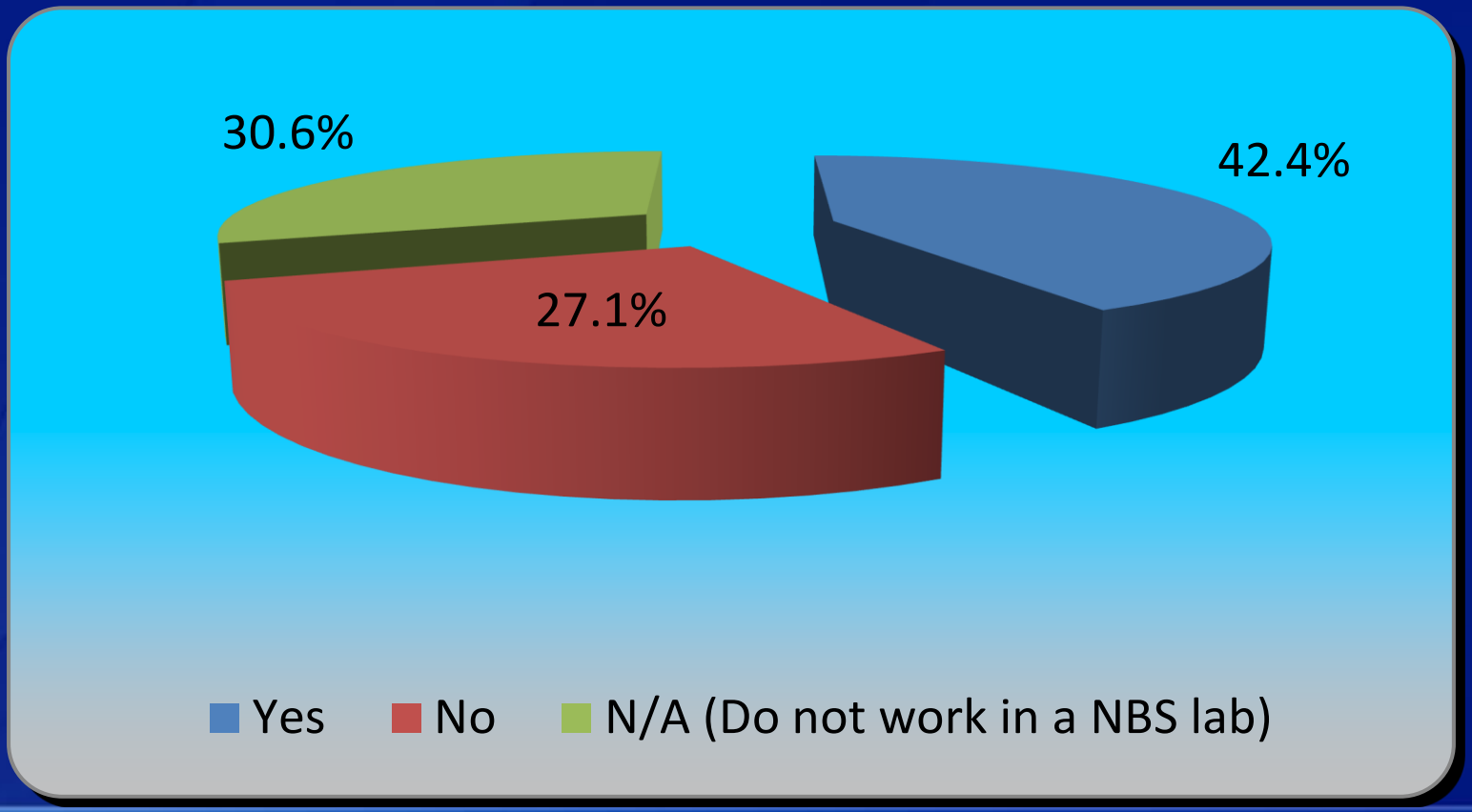
QC Needs of NBS Laboratories
2011 NBS & GT Symposium Poll Results

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



QC Needs of NBS Laboratories
2011 NBS & GT Symposium Poll Results

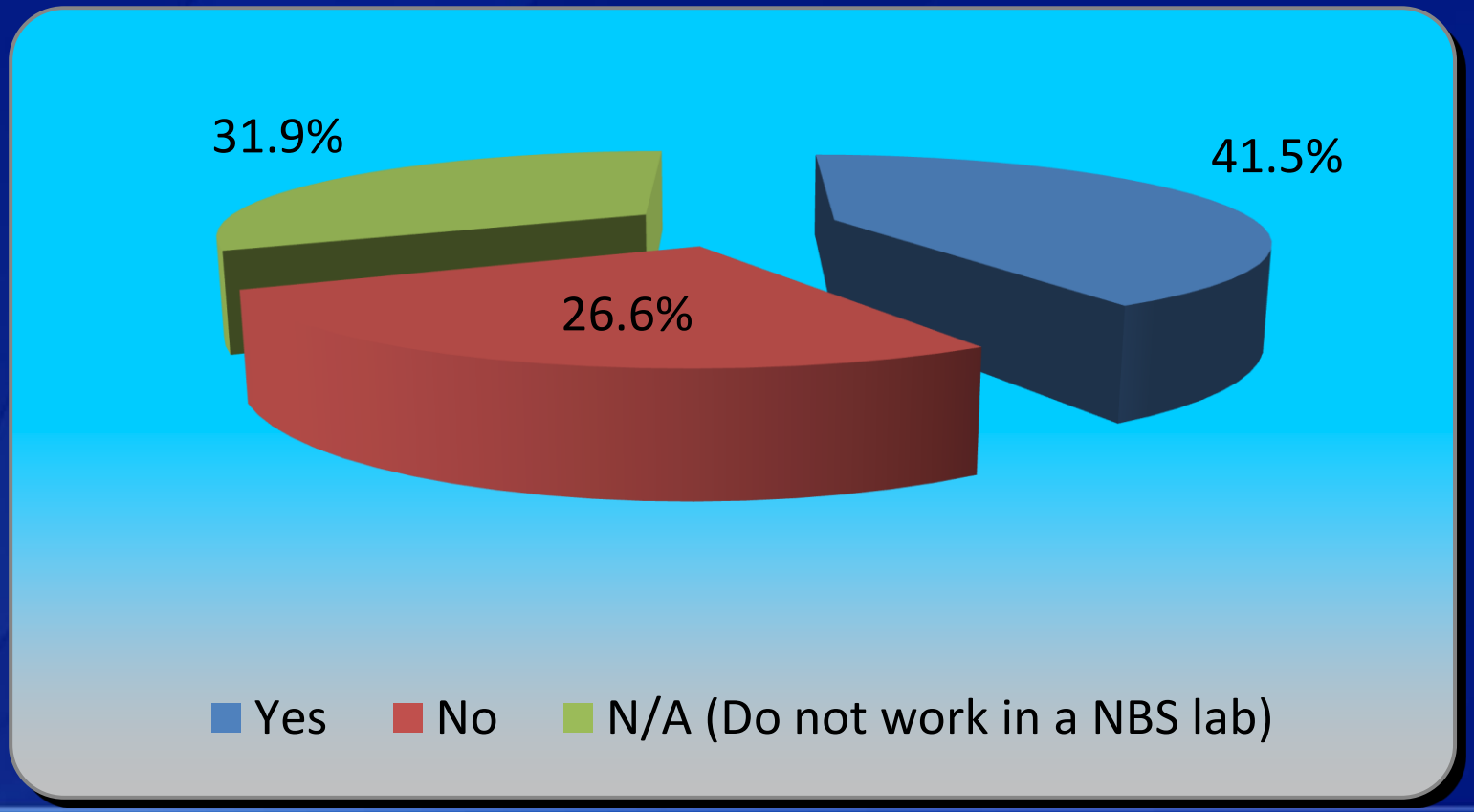
Would your laboratory find a molecular QA for MCAD useful?
N=85



QC Needs of NBS Laboratories
2011 NBS & GT Symposium Poll Results

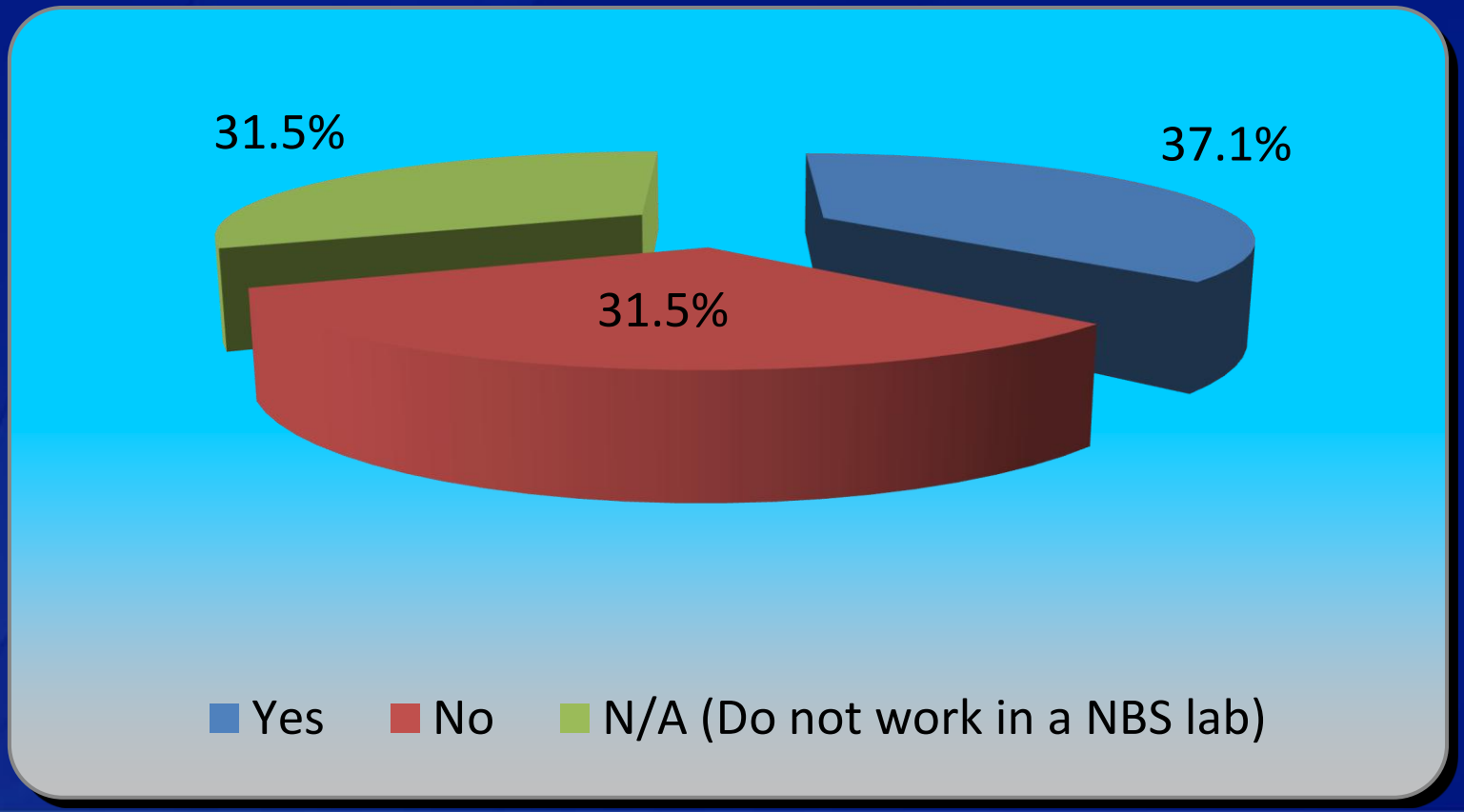
Would your laboratory find a molecular QA for Hemoglobinopathies useful?

N=94



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



Acknowledgements

The Johns Hopkins Hospital

California Dept of Health

University of Wisconsin

Sequoia Foundation

Case Western Reserve University

California CF Centers

NSMBB Branch members

All of our CF donors and NBS PT participants