Cystic Fibrosis Screening: Attempts To Reduce False Negatives → Summary of CLSI NBS05 Addendum (Revisions to CF NBS Guidelines)

Mei Baker, M.D., FACMG

Co-Director, The Newborn Screening Laboratory at WSLH

Associate Professor, Department of Pediatrics

University of Wisconsin School of Medicine and Public Health

The Problem & Challenge*

- Both false negative and false positive results occur in CF NBS with any of the current algorithms.
- As in other NBS tests, while false positives are manageable, the false negatives (“missed cases”) are construed as a failure of the screening program
- All CF NBS strategies begin with IRT
- Most false negatives are due to IRT levels below the cutoff values being used in the various methods, although some are due to incomplete CFTR panels

*Therrell et al. Immunoreactive trypsinogen as a biomarker for cystic fibrosis:challenges in newborn dried blood spot screening. Mol Genet Metab. 2012; 106:1-6.)

Clinical and Laboratory Standards Institute (CLSI)

MISSION: To develop **best practices** in clinical and laboratory testing and **promote their use** throughout the world, using a **consensus-driven process** that balances the viewpoints of industry, government, and the healthcare professions.

CONTRIBUTION TO CF: Developed 2011 Guidelines for CF NBS

CLSI Working Group on CF NBS

Goal: Revise the 2011 Guidelines, as planned

"Despite its widespread use, NBS-CF is complicated by a number of issues regarding laboratory science and public health application. This has led to highly divergent approaches for NBS-CF, some of which may not provide the best utilization of laboratory technology nor the optimal public health effectiveness."

CLSI Addendum: Rationale

In 2011, *it was recognized that CF newborn screening was still in a state of evolution and that some aspects could not be addressed definitively.*

Need for an *evidence-based modification to the CF NBS Guidelines* published in 11/11 to cover:

- ***reassessment of IRT cutoff value recommendations and the use of floating rather than fixed cutoff value;***
- ***evaluation of recent large study data on the potential added value of PAP and development of international consensus on the use of this analyte; and***
- ***potential revision of recommendations regarding CFTR panels, using information from the CFTR2 project and taking into account new biotechnologies such as next generation sequencing.***

CLSI NBS05 (I/LA35) Addendum Development Working Group

- Chairholder & Vice-Chairholder
  - Philip Farrell, MD, PhD (University of Wisconsin)
  - Olaf Sommerburg, PD Dr. Med. (University Children's Hospital Neuenheimer Heidelberg)
- Voting Members
  - Gary Hoffman (Wisconsin State Laboratory)
  - Annika Hiekkanen, M.Sc (PerkinElmer, Diagnostics)
  - Enzo Ranieri, PhD (Women's and Children's Hospital Australia)
  - Marci Sontag, PhD (University of Colorado)

CLSI NBS05 (I/LA35) Addendum Development Working Group

Contributing Advisors/Reviewers:

- Patrick Sosnay, MD (Johns Hopkins University)
- Marie Early, PhD (CDC)
- Martin Kharrazi, PhD (California Department of Public Health)
- Richard Parad, PhD (Harvard Medical School)
- Mei Baker, MD (University of Wisconsin)
- John Thompson, PhD (Washington State Department of Health)
- Kevin Southern, Mb ChB, PhD (University of Liverpool Children's Hospital)
- Susanna McColley, MD Lurie Children's Hospital, Northwestern University)
- Elinor Schwind (Beth Israel Medical Center)
- Carlo Castellani, PhD (Azienda Ospedaliera, University of Verona)
- Audrey Tluczek, RN, PhD (University of Wisconsin Madison)

CLSI Guidelines: 2011* & 2014-15 (see NBS05 document)

- Re- IRT/IRT: NOT recommended
- Re- CFTR2 and CFTR panels for IRT/DNA: include only CF-causing mutations in expanded panels
- Re- PAP: may be useful for IRT/PAP & as an adjunctive test to reduce the detection of carriers
- Re- communication/counseling: needs improvement
- Re- sweat Cl testing: essential for confirming Dx

CLSI Revised Recommendations on IRT*

- ***The IRT/IRT only strategy is not recommended for screening in regions with adequately defined CF-causing mutations and adequate resources, unless legal barriers exist. For current IRT/DNA algorithms in which a high IRT and at least one mutation leads to sweat test, adequately defined should be interpreted as at least 90% and preferably 95% of CF-causing alleles identified in the existing population of CF patients, which would lead to only 0.25-1% of cases missed because of a failure in the DNA tier.***

- ***To improve further the use of this important biomarker, more research is needed on assay methods and on the issue of fixed vs floating cutoffs in geographically and climatologically diverse regions.***

Tentative recommendations, expected to be approved in 1/15.

Sontag et al. Improving the Sensitivity and Positive Predictive Value in Cystic Fibrosis Newborn Screening using IRT/IRT/DNA: Four States' Experience*, submitted 2014.

- ***The data revealed that employing a cutoff of 105 ng/ml (~98.5th percentile) with IRT/IRT would lead to a hypothetical missed case rate of 19.4% in CF infants without meconium ileus compared to a actual missed case rate of 3.0% with the lower cutoff of 60 ng/ml used for IRT/IRT/DNA in Colorado.***

- ***While the range of potential missed cases avoided varied among states employing IRT/IRT/DNA with a range of 9.2% to 24.8% in the three states, it is readily apparent that an unacceptable number of infants would be missed with IRT cutoffs set at 105 ng/ml.***

***** *proved that the false negative rate could be significantly reduced by lowering the IRT cutoff below levels traditionally used in regions employing IRT/IRT*

Carlo Castellani, et al., European Best Practice Guidelines For Cystic Fibrosis Neonatal Screening (JCF 8:153-173, 2009)

"There is little evidence to support the use of IRT alone as a second tier, without involving DNA mutation analysis. However, if IRT/DNA testing does not lead to the desired specificity/sensitivity ratio, a screening program based on IRT/IRT may be used."

Pediatrics, February 2009

Clarification of Laboratory and Clinical Variables That Influence Cystic Fibrosis Newborn Screening With Initial Analysis of Immunoreactive Trypsinogen

Molly Kloosterboer, MS[a], Gary Hoffman, BS[b], Michael Rock, MD[c], William Gershan, MD[d], Anita Laxova, BS[c], Zhanhai Li, PhD[e], Philip M. Farrell, MD, PhD[a,b]

Departments of [a]Population Health Sciences, [c]Pediatrics, and [e]Biostatistics and Medical Informatics and [b]Wisconsin State Laboratory of Hygiene, University of Wisconsin, Madison, Wisconsin; [d]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin

The authors have indicated they have no financial relationships relevant to this article to disclose.

What's Known on This Subject

CF newborn screening has been performed by using IRT analyses as the initial step, and 49 states are now screening by using either the IRT/IRT or IRT/DNA. Although variations in IRT levels occur, the factors and their importance have not been delineated.

What This Study Adds

This study clarifies the causes of IRT variations and demonstrates their significance as related to CF diagnosis. Our data reveal marked variations associated with seasonal and assay kit changes and also show that IRT/IRT sensitivity is significantly lower than that of IRT/DNA.

Floating IRT Cutoff Will Reduce False Negatives (Kloosterboer et al *Pediatrics* 2009)

- ***The sensitivity of the IRT/DNA-multimutation protocol changed from 90.6% (95% CI: 82.7%–98.4%) with a fixed cutoff to 96.2% (95% CI: 91.1%–100%) with a floating daily cutoff…***

- ***3 newborns had IRT levels that would have been below a fixed cutoff value and, therefore, would have been missed cases, but they were detected because of the WSLH's floating cutoff…***

- ***by using receiver operator curve analyses, we also found that the 96th percentile gave the best combination of sensitivity (96.2%) and specificity (99.8%), compared with the 97th, 98th, and 99th percentiles.***

IRT Floating Cutoff Calculation*

1. The highest 4% of the IRT levels is calculated on all specimens received daily, and no statistically significant variations were found when at least100 specimens were used in the calculation.
2. To avoid skewing by outliers, if there are specimens with IRT levels ≥170 ng/ml (≥ 99.8th percentile), they are removed and replaced by an equal number of specimens with the highest IRT levels from those specimens not included in the original highest 4% calculation.
3. If there are multiple specimens with the same IRT level at the 96th percentile cutoff, they are all included in the 4% list.

*When this method is used and at least 100 specimens are included in the calculation, statistical variability does not preclude daily, accurate determination of a floating IRT cutoff value.

IRT Floating Cutoff Statistics Study Design and Results

- WI IRT data from 2009 & 2010 were evaluated
- N = 69,680 and 67,576
- Daily* or weekly IRT floating cutoff values assessed
- Minimum statistical sample size determined (N =100)
- Optimal floating cutoff centile determined, assessing 95th, 96th (best), 97th, 98th, and 99th
- ROC analysis done to identify best combination of sensitivity and specificity (again 96th percentile)
- Multiples of the median also assessed (less useful)

* Results show that daily floating cutoff calculation is reliable with >100 IRT 's

"Every CF patient should have a sweat test!" (Preston Campbell, MD→ 29 September 14)

Reasons Why Sweat Testing is Essential to Confirm Dx:

1. IRT/DNA & other NBS = screening tests (NOT Dx)
2. QNS challenge is no excuse to "avoid" sweat test
3. With 2 CF-causing mutations, even F508/F508del, a presumptive "genetic diagnosis" is NOT a clinical or functional diagnosis because:

1) Guthrie cards with NBS blood can be and ARE mislabeled

2) Guthrie cards may have fictitious blood (eg, nurse/tech)

3) CFTR mutations could be in *cis* (ie, on the same chromosome)

Acknowledgements

- Gary Hoffman, BS
- Philip Farrell, MD, PhD
- Zhanhai Li, PhD