Cystic Fibrosis Screening: Attempts To Reduce False Negatives

Summary of CLSI NBS05 Addendum (Revisions to CF NBS Guidelines)

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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.

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.”
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

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- 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.*

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

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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 least 100 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)
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