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of Health**

**Wadsworth
Center**

Newborn Screening: Laboratory Perspective on Cut-off Establishment

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February 9, 2017

Newborn Screening Programs are Regulated

- **Clinical Laboratory Improvement Amendments, 1988 (CLIA)**
- **New York State is CLIA-exempt**
 - **On-site surveys (biennial)**
 - **Review of method validation**
 - **Review of Director's qualifications**
 - **Requirement for proficiency testing**



Newborn Screening Programs Can be Accredited

- **College of American Pathologists (CAP)**
- **Professional Standards:**
 - **Clinical Laboratory Standards Institute (CLSI)**
 - **American College of Medical Genetics and Genomics (ACMG)**
 - **Association of Molecular Pathologists (AMP)**



Definitions 1

- **Fixed Cut-off:** an established value based on the analytical result
- **Floating Cut-off:** an established value based on a percentile (i.e. the top 5%)
- **Algorithm:** flow chart that manages samples in the context of cut-offs
- **Retest:** the same test repeated in duplicate or triplicate on the same specimen
- **Borderline:** a result that is slightly out of range



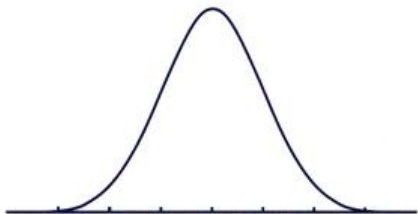
Definitions 2

- **Repeat testing:** the same test repeated on a newly collected specimen; may be after a borderline result
- **Second tier/Reflex:** a different test done in house using the same specimen
- **Tiered testing:** use of a second tier or reflex test
- **Confirmatory Testing:** a different test on a different specimen outside of NBS; purpose is to establish a clinical diagnosis



Newborn Screening Is Not Diagnostic

- **Partnership with families, providers, and specialists**
- **Risk assessment: Identify infants at increased risk**
- **High throughput**



- **Identifies spectrum of disease**
- **Case definitions essential!!**
- **Takes all comers**



Diagnostic v. Screening

- Consider babies are asymptomatic
- Accept false positives
- Estimate of risk level
- Requires confirmatory testing
- Screening misses some infants; report language pointing to baby's clinical picture
 - Mammograms have a 20% false negative rate
 - Screening v. Diagnostic Colonoscopy
 - Glucose / Cholesterol



Screening is Simple and Complex

- **Mandate can impact experience**
- **High throughput**
- **Assessing for rare events/conditions**
- **Redundant equipment**
- **Reagents**
- **Dependent on state population**
- **Temperature**
- **Time of year**



Screening is Simple and Complex

- **A baby's overall metabolism is dependent on:**
 - **Baby's birth experience**
 - **Baby's biology**
 - **Baby's feeding**
 - **Gestational age**
 - **Birthweight**
 - **Underlying medical conditions or treatments**
 - **Underlying maternal conditions (reported or unreported)**
 - **Gender**
 - **Race / Ethnicity**



CLIA is Silent on Validation Methods 1

- **Matrix effects**
- **Interference**
- **Linearity**
- **Limit of Quantification, Upper Limit of Linearity; Limit of Detection**
- **Precision and Accuracy (Reproducibility / Recovery)**
- **Carryover**
- **Specificity**
- **Method Comparison**
- **Multiple Instrument Comparison**
- **Verification**



CLIA is Silent on Validation Methods 2

- **Establish cut-off**
 - select number of normal specimens to screen
- **Statistical analysis**
 - mean and standard deviation
 - select a range (3X to 6X SD)
 - establish a percentile cut-off
 - compare to community
 - examine positive rate
- **Continuous quality improvement**



Positive Controls

- **Work with advocates; referral centers; other states**
- **Consent to use specimens**
- **IRB approval required**
- **Availability of ‘real’ controls; adults v. babies**
- **Heterozygous controls**
- **Synthetic controls**



Quality Control / Quality Assurance

- Mechanism to use de-identified specimens – can vary based on state of field
- Need to ensure positive controls available
- States precluded from storing samples
- Newborn Screening Quality Assurance Program
 - Quality assurance materials
 - Reference materials
 - System for proficiency testing
- Continuous quality improvement
- NewSTEPs and community experience



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What Constitutes a “Result”

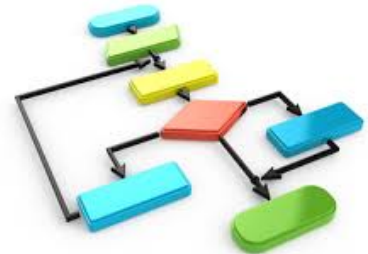
- Primary analyte result
- Secondary analyte result
 - Can be other analyte(s) results
 - Can be ratios
- Baby factors (age, birthweight)
- Retest result
- Second-tier test result
- Is the specimen ‘initial’ or ‘repeat’?

Clinical expertise must then be used to consider family history and any later symptoms



MCAD as an Example

- C8 (1* marker) ≥ 0.8 : -- referral
- C6 (1* marker) ≥ 0.5 : -- referral
- C8 / C2 (2* marker):
 - Referral: $0.35 \leq C8 < 0.80$ and $C8 / C2 \geq 0.05$
 - DNA done as an adjunct
- Borderline = ask for repeat specimen
- $0.30 \leq C6 < 0.50$
- $0.35 \leq C8 < 0.80$ and $C8 / C2 < 0.05$
- 2 borderline results constitute a referral
- We don't refer on ratios alone



NBS is Subject to CLIA Rules

§493.1253 Standard: Establishment and verification of performance specifications (b)(1)(i)(B) Precision. Interpretive Guidelines §493.1253(b)(1)(i)(B) Precision (Reproducibility) - The laboratory is responsible for verifying the precision of each test system by assessing day-to-day, run-to-run, and within-run variation, as well as operator variance. This may be accomplished by:

- **Repeat testing of known patient samples over time;**
- **Testing QC material in duplicate and over time; or**
- **Repeat testing of calibration materials over time.**



Thoughts to Consider

- **Mechanisms for constant physician education**
- **Ensure notes / disclaimers are transmitted to the electronic record**
- **Consider more information on reports**
- **Standardize methods of validation**
- **Forum for sharing CQI**
- **Ensure missed cases are reported back for investigation**
- **Ensure follow-up is linked to the laboratory**
- **Ensure a case is a case -- definitions**





Thank you for your attention!



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