Newborn Screening: Laboratory Perspective on Cut-off Establishment

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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
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 ≤ C8 < 0.80 and C8 / C2 ≥ 0.05
  - DNA done as an adjunct

- Borderline = ask for repeat specimen
- 0.30 ≤ C6 < 0.50
- 0.35 ≤ 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!