Applying Quality Standards to Biochemical Genetic Testing

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Faculty Disclosure

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“Nothing to disclose”
Objectives

• Address the unique challenges of biochemical genetics testing compared to other types of clinical laboratory testing

• Describe the application of general quality guidelines to specific biochemical genetics tests including amino acid analysis and enzyme assays
What is Biochemical Genetics?

- Specialized testing for inborn errors of metabolism
- High-complexity testing governed by CLIA, but not recognized as a CLIA specialty or subspecialty
- Important features distinct from other laboratory sections
  - All are laboratory developed tests (LDT), none are FDA approved or cleared
  - Testing often relies on recognition of metabolic patterns, not just single analytes
  - Results depend on and are interpreted in the context of clinical history
Laboratory Testing: The Big Picture

1. Test ordering
   Sample Collection

2. Transport

3. Receiving in preanalytical area
   Accessioning into LIS
   Aliquoting, labeling

4. Sample prep
   Testing (manual or automated)
   Data analysis and calculation

5. Results interpretation
   Reporting in LIS
   Generating and transmitting report
Clinical Laboratory Improvement Amendments (CLIA)

The Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations include federal standards applicable to all U.S. facilities or sites that test human specimens for health assessment or to diagnose, prevent, or treat disease. CDC, in partnership with CMS and FDA, supports the CLIA program and clinical laboratory quality.


Clinical Biochemical Genetics Checklist

CAP Accreditation Program
Specific areas related to BG testing:

- Establishing test performance (April 8)
- Quality assurance (March 4)
- Proficiency testing
- Personnel qualifications and training
Quality Assurance: 42 CFR §493.1256

• The lab must have **control procedures** to monitor accuracy and precision, and to detect immediate errors from test system failure, adverse conditions, or operator performance.

• If control materials are not available, the laboratory must have an **alternative method** for detecting immediate errors and monitoring test system performance over time.
Regulation and Guideline Examples

**CLIA**
- “…Calibration using criteria verified or established by the laboratory…”

**CAP**
- “…Appropriate calibration or calibration verification is performed on each day of patient testing…” (QC material may be used to verify)

**MMWR**
- “…Each laboratory preparing these materials should ensure their validation, including verifying each new batch against an old batch, and ensure that appropriate calibration and calibration verification procedures are in place.”
Applying MMWR Guidelines:

Specific Examples

• Amino acid analysis
• Liquid chromatography/mass spectrometry assays
  – Amino acids, acylcarnitine profile (LC-MS/MS)
• Enzyme assays
Quantitative Amino Acids
Amino Acid Analysis: Quality Issues

- Calibration and quality control
- Linearity and AMR
- Dilution criteria
- Chromatographic characteristics
- Carryover detection
Amino Acid Analysis: Quality Issues

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Calibration and Control Material

• Commercial sources of amino acid mixtures*
  – Examples: Pickering, Sigma, Wako
  – Concentrations not always as advertised
  – Mixtures missing some unstable compounds (e.g., gln, tryp)
  – Control and calibration material must be prepared separately

• Acceptable QC material
  – Standard mixtures
    • Matrix effects must be evaluated or documented
    • Traditional analyzers (e.g., Biochrom) are matrix insensitive
  – Patient samples
    • Undiluted, pooled, spiked

* References to trade names do not imply endorsement and are given for informational purposes only
Establishing QC Thresholds

- No guidelines for multiple analyte tests
  - Each lab must define according to own method performance
- Example:
  - Prepare QC material, run “multiple” times
  - Establish QC limits, for example:
    - ± 10-15% of mean
    - ± 2 SD of mean
- Evaluate new batch of QC material next to the current batch before putting into production
Frequency of QC Runs

**CLIA**
- Each day that testing of patient specimens is performed, at least two controls of different concentrations must be included...

**CAP:**
- Two controls at two concentrations must be run daily or with each batch of samples/reagents...

Two control runs every 24 hours may not be practical for traditional amino acid analyzers
QC Frequency Guided by MMWR

Every 24 hours:

- Testing one control * AND
- Spiking patient specimens with at least one internal standard

* Acceptable control material = Mixed-level or single-level control, or previously tested specimen

Batches > 24 hours:
Bracket with control samples at the beginning and end of the run

Batches > 48 hours:
Insert control sample in the run every 24 hours and spike patient specimens with at least one internal standard.
Evaluating QC Data

• How many compounds should be monitored?
  – All reported compounds
  – A subset of compounds based on available standards

• What if some values fall outside of QC limits?
  – No single best answer; criteria for acceptance should be established and documented

• What QC statistics should be monitored?
  – No statistics specified; example = analytic imprecision (SD, CV)
  – Plotted to detect trends
AMR Verification

• Analytical measurement range = range of values that a method can directly measure without pretreatment (e.g., dilution) that is not part of the usual assay process

• Verify using matrix-appropriate materials
  OK to use standard material if no matrix effects

• Include the low, mid and high range of the AMR at a minimum

• Define appropriate acceptance criteria, document every 6 months
Dilution Criteria

- AMR defines upper limits of reportable values
- Samples with values exceeding the AMR must be diluted
- Dilution procedure must be documented
  - Diluent, maximum allowable dilution
  - Use knowledge of clinically feasible values to define
Dilution Example

The AMR for leucine has been validated up to a target of 2,000 nmol/ml

An MSUD patient has a leucine of 2,500 nmol/ml

Possible options (may vary with clinical situation):
  - Report as >2,000 nmol/ml
  - Dilute and rerun
  - Report as preliminary, then dilute and rerun
Quality Issues: Chromatography

• Chromatographic characteristics
  – Retention time
  – Internal standard abundance
  – Peak shape
  – Column backpressure

• Validating a new column
  – Run previously analyzed samples and QC material
  – Compare performance to pre-established criteria

• Carryover detection
  – Run a blank after high control
  – Re-inject any patient following a very high result (define criteria)
LC-MS/MS Assays

![LC-MS/MS Assay Image]
Quality Issues: LC-MS/MS Methods

• Instrument performance
• Ion suppression
  – From sample matrix, associated with electrospray ionization
  – Monitor abundance of internal standards
• Testing blank samples
  – Monitoring background (solvent blank)
  – Monitoring interference from entire system (processed blank)
• Carryover detection
• Chromatographic characteristics
Mass Calibration and Tuning

• Calibration
  – Assigns accurate masses to specific ions for accurate detection
• Tuning
  – Defines MS/MS parameters for optimized sensitivity
• Frequency
  – According to vendor recommendations
  – After major preventative maintenance or instrument failures
Enzyme Assays
Enzyme Assays

- Controlling for specimen integrity
- Calibration and analytical QC
- Controlling for assay conditions
- Test interpretation and reference ranges
- Proficiency testing
Controlling for Specimen Integrity

• Loss of activity during shipping and processing
  – Shipment control (e.g., unrelated individual, parents)
  – Monitor specimen quality upon arrival
  – Define criteria for acceptance or rejection

• Loss of activity from cell disruption (freeze-thawing, sonication)
  – Include normal sample through entire process
  – Define other QC indicators to monitor loss of activity (e.g., testing an unrelated enzyme)
Calibration and Analytical QC

• QC similar to other quantitative assays
• Calibration, AMR verification required for the analyte being measured (reaction product)
• Monitoring performance of assay controls
• Define in SOP and document
• Full range calibration must be performed at least twice/year
Assay Controls: General Considerations

Ideally include a positive and negative control with each batch

MMWR: Controls and reference material should be selected based on:

- Patient population
  - e.g., age-matched if appropriate
- Prevalence of disease
  - rare, authentic positives may not be available
- Purpose of the test
  - e.g., heterozygote controls for carrier testing
Assay Controls: Examples

- Pooled specimens from normal, affected or carrier individuals
  - Aliquot and freeze
- Heat-inactivated sample
- Repeat testing of previous patient
- Testing an unrelated enzyme
- Testing enzyme from another source (e.g., cultured fibroblasts, *C. elegans*)

- QC acceptance criteria and performance monitoring
Controlling for Assay Conditions

• Evaluation and monitoring of reagents and substrates
  – QC performance
  – Testing of blank samples

• Monitoring of instruments and equipment
  – Thermometers
  – pH meter
  – Spectrophotometer
  – Fluorometer
Proficiency Testing

- PT required for all clinical assays including enzyme tests
- Limited number of external PT programs for enzyme assays
  - NTSAD (Tay-Sachs carrier testing)
  - ERNDIM (lysosomal enzymes)
  - CDC NSQAP (lysosomal enzyme newborn screening)

- Alternative performance assessment options (detailed by CLSI)
  - Interlaboratory exchange
  - Repeat testing of blinded samples
  - Exchange with a research facility or international laboratory
  - Interlaboratory data comparison
CAP Sample Exchange Registry

- Service to connect laboratories performing testing with no formal PT available
- Labs submit testing information and materials to registry
- CAP distributes samples to all participating labs
- CAP compiles results and returns a summary report to participants
- Currently available for Biotinidase and GALT testing
PT: Special Consideration for Enzymes

- Quantitative results for many enzyme assays vary widely from lab to lab

- PT should focus on reaching the correct diagnosis rather than assessment of quantitative performance
In Summary

• Biochemical Genetics Laboratories must ensure quality performance throughout the entire testing process

• All QC and QA procedures must adhere to CLIA regulations and be thoroughly documented

• Laboratories have flexibility in the development and implementation of these procedures depending on workflow, instrumentation and other site-specific issues

• MMWR relates specific CLIA regulations to the unique setting of biochemical genetics
Biochemical Genetic Test
Establishment and Verification

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