## Beyond Westgard Rules: Quality Control for Mass Spectrometry

Dr Russell Grant  
VP R&D  
Laboratory Corporation of America® Holdings.

### Assay Validation Criteria and Acceptance

<table>
<thead>
<tr>
<th>Validation Process</th>
<th>Materials Requirements and Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy (Intra and Inter-assay)</strong></td>
<td>Min. 2 levels of spiked QC’s including LLOQ and ULOQ; Min 20 measurements each in a single run and across 25 days. Bias % &lt;15% or 20% @ LLOQ.</td>
</tr>
<tr>
<td><strong>Precision (Intra and Inter-assay)</strong></td>
<td>Min. 2 levels of spiked QC’s including LLOQ and ULOQ; Min 20 measurements each in a single run and across 25 days. CV % &lt;15%/20% @ LLOQ.</td>
</tr>
<tr>
<td><strong>Standard Curve Fit</strong></td>
<td>6-10 levels run in each assay, evaluated over 5 assays (days), R=0.985, Slope CV&lt;15%</td>
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<tr>
<td><strong>Drug Selectivity</strong></td>
<td>Up to 250 potentially interfering endogenous and exogenous analytes spiked supraphysiological concentrations, Response &lt;20% @ LLOQ when spiked at Maximum Normal x10 ideal, minimally &lt;20% LLOQ @ upper normal range.</td>
</tr>
<tr>
<td><strong>Materials Selectivity</strong></td>
<td>Blank matrix test 8 or more lots Response&lt;20% LLOQ in 5 of 8.</td>
</tr>
<tr>
<td><strong>Matrix Interference</strong></td>
<td>Effect of Spd. (icterus and Hemolysis). Bias % &lt;15%/20% @ LLOQ. Net anticoagulants Bias % &lt;15%/20% @ LLOQ. Alternate sample types: Deming regression 0.9-1.1, R &gt;0.9 for same reference interval. Matrix effects (sample mixing) Bias&lt;15%</td>
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<tr>
<td><strong>Carry-over</strong></td>
<td>After ULOQ – 3 runs including &lt;20% LLOQ, Stress test (10-100x ULOQ), how many for &lt;20% LLOQ.</td>
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<tr>
<td><strong>Stress test (10-100x ULOQ)</strong></td>
<td>How many for &lt;20% LLOQ</td>
</tr>
<tr>
<td><strong>Dilutional Linearity</strong></td>
<td>5 Level sample mixing - Accuracy % 80% to 110% Serial 100-20x dilution - Accuracy % 80% to 115%</td>
</tr>
<tr>
<td><strong>Sample Stability</strong></td>
<td>All conditions tested from amino to final result Mean Bias = 15%-20% @ LLOQ, %CV = 15%-25% @ LLOQ. Deming regression 0.9-1.1, R &gt;0.9.</td>
</tr>
<tr>
<td><strong>Inter-assay Comparison</strong></td>
<td>Min 20 – Internal/Inter-assay tests – For information only Internal assays same calrs – Deming regression 0.9-1.1, R &gt;0.9 Same assay different system – Deming regression 0.95-1.05, R &gt;0.9 Same assay Manual/Automation – Deming regression 0.85-1.05, R &gt;0.9</td>
</tr>
<tr>
<td><strong>Reference Interval</strong></td>
<td>20 (95% CI) for range transfer L=20 outside existing range</td>
</tr>
<tr>
<td><strong>Transition ratio monitoring</strong></td>
<td>Bias from extracted calibrators in each batch &lt;20% up to 3x LLOQ &lt;15% after that for ratio&lt;0.5</td>
</tr>
<tr>
<td><strong>Batch Size</strong></td>
<td>Maximum 184 samples prior to recalibrate. Bias % &lt;15%/20% @ LLOQ, %CV % &lt;15%/20% @ LLOQ. Carry-over &lt;15% @ LLOQ. Repeat samples n = 2 (first and 2nd plate) Deming regression 0.9-1.1, R &gt;0.9</td>
</tr>
</tbody>
</table>
## Verification Process

### Materials Selectivity
Blank matrix <20% LLOQ in 5 runs.

### Standard Linearity
All Cals used, Accuracy % 85% to 115%, 80 - 120% @LLOQ and R=0.95, Slope CV<5%

### Carry-over
After ULOQ - 3 runs including <20% LLOQ

### Precision (Intra and Inter-assay)
- Total 25 QCs over 5 runs %CV <15% or 20% @LLOQ
- Stress test (10-100x ULOQ), how many for <20% LLOQ

### Inter-assay Comparison
Minimum n=20 per batch – Deming regression 0.90-1.00, R >0.90

- Do not use best technicians for this step
- Add multiple columns/materials lots / systems, time to emulate reality

---

## Assay Translation (R&D – Ops)

### (Assay Viability – QC Ranges Generation n=25 over 5 runs)

<table>
<thead>
<tr>
<th>Assay Translation (R&amp;D – Ops)</th>
<th>(Assay Viability – QC Ranges Generation n=25 over 5 runs)</th>
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<tbody>
<tr>
<td>Plasma Metanephrine</td>
<td>Slope = 1.148</td>
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<tr>
<td></td>
<td>R = 1</td>
</tr>
<tr>
<td>Plasma Normetanephrine</td>
<td>Slope = 1.128</td>
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<td>R = 1</td>
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## Internal QC for LC-MS/MS

### LC System
- Power Failure
- Leak detection
- Pressure beyond pumping
- Air bubble
- Column Degradation
- Complete Sample volume injected
- Injector blockage
- Contamination

### MS System
- Power Failure
- Gas failure
- Temperature effects
- Contamination
- Resolution change
- Sensitivity shift
- Selectivity

### Internal QC Action
- Shutdown (without UPS)
- Shutdown
- Shutdown or No monitoring
- No monitoring
- No monitoring
- No monitoring

---

## Assay Translation (R&D – Ops)

### (Check Manufacturing Process)
System Suitability Test (SST) – “The Doctor”

Goal – System check prior to analysis to confirm appropriateness of test system for analysis or DRIFT

Design:
- Neat solution stored under stable conditions – preferably at the assay LLOQ
- Acquired correctly?
- Retention time – <5% variance
- Sensitivity – S/N > 20:1
- Peak shape – As 0.8-1.2
- Multi-channel acceptance – column-column/channel-system-system

SST is key part of troubleshooting experiments
Ignore the first (or second) injection

HPLC Pressure Trace – “The Heartbeat”

Goal – Pre-batch and per-injection check of appropriate LC performance

Design:
- SOP Example – Annotate an acceptable pressure trace
- Pressure profile – Start and overall maximum pressure
- Changes in pressure profile –
  - Air bubbles in A or B
  - Dead volume differences
  - Column dimensions change
  - Full injection
  - Injector/filter/column/lines blockage
  - Post column blockage (bypass/source probes)

Internal QA – Pre-Batch

LC Solvent Contamination
- Blank, Clean
- Epinephrine LLOQ
- Contaminant
- Resolved

Air Bubble/Column or MP Degradation/Injector blockage
- Air Bubble SST
- SST Day 1
- SST Day 4
- SST Day 4 post LC flush/flush MPB
Internal QA – Pre and During Batch

**MS System Source contamination Pre-run**

- HVA, VMA, 5-HIAA
- 1st Sample, source fouling

**LC-MS/MS Column Degradation RT3 SST at LLOQ**

- SST at LLOQ - Acceptable
- Resolution in underlying RT3 of T3 from reverse T3

---

Internal QA – Pre-Batch

**Checking Instrument Sensitivity with SST at LLOQ**

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<th>Date</th>
<th>Original Tr</th>
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<th>D3</th>
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<th>SST C D2</th>
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<td>226</td>
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</table>

Batch Data Review

**Formula #1 = Tr analyte / Tr internal standard, as a percentage**
Internal QA – LC-MS/MS Batch/Assay Review

**IS Contamination**

- DHEA Double Blank
- DHEA Blank with IS
- DHEA LLOQ 20ng/dL

Sample recovery/injected

- Androstenedione Cal. 5
  - Expected = 250
  - Measured = 299.322
  - Accuracy (%) = 119.729

Internal QA – LC-MS/MS Batch/Assay Review

**MS System Sensitivity drift**

- Phenylalanine D5 IS Plot, IS increase at 97th aqueous calibrator injection (intra-assay inaccuracy batch, n=20 at 8 levels)

MS System Source contamination within Run

- LLOQ for allo-isoleucine (A, 100nm/L)

Internal QA – LC-MS/MS Batch/Assay Review

**Matrix Effects in Individual samples – Signal:Noise**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Conc</th>
<th>RT</th>
<th>Analyte/Peak Area</th>
<th>IS Response</th>
<th>Calc Value</th>
<th>IS Ratio</th>
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</table>
Internal QA – LC-MS/MS Batch/Assay Review

Selectivity Variance - Matrix Effects

D3-Metanephrine IS at 4.3 e5 cps

D3-Metanephrine IS at 1.7 e5 cps

40% of earlier sample

Partial Suppression from co-elutor

Selectivity Variance - Internal Standard Transition ratio plots

Internal QA – LC-MS/MS Batch/Assay Review

Transition Ratio Monitoring – Exploring Outliers

LLOQ sample

Calculated Conc 1.9ng/dL

Concentration 5.3ng/dL

Internal QA – LC-MS/MS Batch/Assay Review

Transition Ratio Monitoring – Exploring Outliers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Conc RT</th>
<th>Analyte Peak</th>
<th>IS Response</th>
<th>Ln. Conc</th>
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</tbody>
</table>
External QC 3-Plex Linked Analytes Plasma Metanephrines
The importance of Automation

Manual Preparation First Run with SPE
Automated First Run With SPE

Manual Preparation
Automated Preparation

Recovery Variance ADDS to Imprecision which makes Westgard Rules Impossible to apply
Use Automation OR Train Technicians VERY well

External QC - Calibrator Stability

Cocaine Profile
ULOQ (1000 ng/mL)

LOQ (1 ng/mL)

Plasma QC (5 ng/mL)

Urine QC (100-110 ng/mL)

Organic QC (105-110 ng/mL)

Blood QC (90-105 ng/mL)

Plasma QC stability at 2-8°C inc 1% NaF
Need Alternate Matrix to assess Calibrator Stability

Column Verification
CAP Checklist, Gas Chromatography and High Performance Liquid Chromatography Section

CHM.16850 Column Verification Phase II
New columns are verified for performance before use.

Evidence of Compliance:
✓ Written procedure for column verification
✓ Records of column verification

“The 3 R’s”: Verifiable Column Performance Characteristics

Retention – Are all analytes properly adsorbed to the new stationary phase
Resolution – Are all analytes exhibiting appropriate desorption from the new stationary phase
Response – Are any components of the new column affecting MS ionization efficiency
Verification Procedure:

1. System Suitability Test Solutions (At concentrations slightly higher than the assay’s extracted LLOQ)
2. Replicate Injections (More than 3)
3. DO NOT USE THE FIRST INJECTION (or the second, and possibly the third, dependent on the particular column pass/failure required). Set the appropriate limits.
4. Have confidence in a working system prior to column verification run

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<th>Direct Observations</th>
<th>Calculated Observations</th>
<th>Criteria</th>
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Verification Data

Multi-analyte panel, API 3200 (Scheduled MRM) Col Verification, SST Injection #3

Verification Data, New Guard Column

Multi-analyte panel, API 3200 (Scheduled MRM) Col Verification, SST Injection #3 Post-Guard Column Replacement
When Accuracy based QC isn’t accurate

External Accuracy based mixture (~50 analytes) in Phenol/water

Supra-physiological ~250uM

Measure Neat (no IS), Neat diluted (class A)

Determine how to deploy “accuracy” based materials that do not represent the clinically observed concentrations of mixtures

Inter-Laboratory Correlation LabCorp and St Pauls, BC

Concentrations < 45ng/dL

N=52

Intercept: -0.096
CI Intercept: [-0.557, 0.488]

Slope: 0.985
CI Slope: [0.954, 1.014]

R-squared: 0.990513

Mean Difference: -2.49%
SD Difference: 5.304%

Acknowledgement: Dr Daniel Holmes,
Grace Van Der Gugten

Automated Data review

If [examined value] exceeds [a threshold], then flag with [text string].

<table>
<thead>
<tr>
<th>Calibration Concentration</th>
<th>Intra-Batch Variability</th>
<th>Inter-Batch Variability</th>
<th>Quality Control</th>
<th>flagged if exceeded by</th>
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<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>threshold</td>
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</table>

- Quality Control: determined from instrument
- flagged if exceeded by threshold
Acknowledgements

Patricia Holland, Matt Crawford, Stacy Dee, Mary Morr, Dr Marcia Eisenberg, Dr Walt Chandler

Brian Rappold

Randy Julian

Dr Andy Hoofnagle