Optimizing the Post-implementation Monitoring of a LC-MS/MS Method

Julianne Cook Botelho, PhD
Centers for Disease Control and Prevention
Atlanta, GA

AACC- Mass Spectrometry in the Clinical Lab:
Best Practice and Current Applications
Tuesday, September 17, 2103

National Center for Environmental Health
Division of Laboratory Sciences

Disclosures

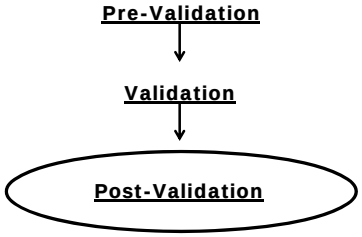
- **Nothing to disclose**
- **Any mention or allusion to specific vendors or vendor parameters should not be seen as endorsement**
- **Some aspects of CLSI C60(to be renamed C62) Liquid Chromatography-Mass Spectrometry Methods; Draft Guideline are included in this presentation**
- **CLSI Guideline C60 is still under development and may not represent the final guidelines**

Learning Objectives

After this presentation, you should be able to:

1. **Identify what post analytic monitoring is and why it is necessary**
2. **Describe how post analytic monitoring can be used to evaluate the performance of your LC-MS Instrument**
3. **Explain how QC materials and other parameters can be used to confirm sample analysis performance**
4. **Identify how proficiency testing (PT) programs can be used in combination with assessments to monitor assay performance over time**

What is post-implementation monitoring?



Approach to verify test method performance specification during routine analysis

Goals and Objectives of Post-implementation Monitoring

- **Assure validity of measurements**
  - Confidence in reported values
- **Identify risk of potential problems**
  - Minimize, identify, and correct analytical errors in real time
  - Ensure optimum LC-MS/MS and method performance
  - Less downtime, increased productivity
- **Compliance**
  - CLIA, FDA

Parameters to monitor

Parameter	Tools
Instrument Performance	System Suitability Sample (SSS)
Sample Analysis Performance	Quality Control Materials (QC) and additional parameters
Assay Performance Over Time	Proficiency Testing (PT), Accuracy Assessments, Lot to Lot, and Instrument Comparisons

Parameters to monitor

Parameter	Tool
Instrument Performance	**System Suitability Sample (SSS)**
Sample Analysis Performance	Quality Control Materials (QC) and additional parameters
Assay Performance Over Time	Proficiency Testing (PT), Accuracy Assessments, Lot to Lot, and Instrument Comparisons

Parameters of SSS to monitor for instrument performance

Parameter
Shift in Retention Time (RT) • gradual or sudden
Peak Shape • Tailing, Fronting, Asymmetry
Additional Peaks
Drop in peak intensity • overall drop (gradual/sudden) • Increase in background noise
Change in Analyte:IS Ratio

Monitor in real time and over time

Acceptable criteria set during validation

SSS- Sample Characteristics

- **Type of Sample**
  - non-extracted sample (analyte and IS)
  - Does **NOT** go through sample preparation
  - Concentration Suggestions-
    - near LLOQ to evaluate instrument sensitivity
    - middle of analytical measurement range
    - near a medical decision point

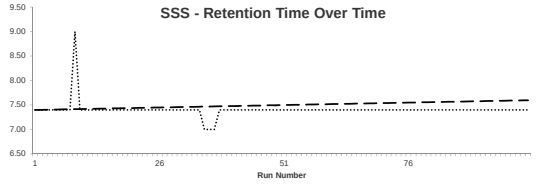
Prepare a large batch and store according to stability standards

SSS- Application for Instrument Performance Evaluation

Use of SSS	Purpose	Frequency
Before Analysis	Confirm instrument performance	Before the start of a run
During a run	Confirm and monitor performance of instrument during the entire run	Depends on stability of instrument; if changes in parameters are common consider more injections
After instrument maintenance, power outage, break in vacuum, tuning/calibration	Confirm instrument is within performance specifications and stable prior to sample analysis	Multiple injections Approx. 5-10

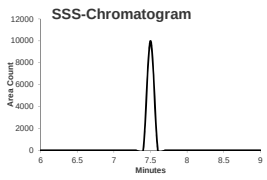
SSS Shift in RT

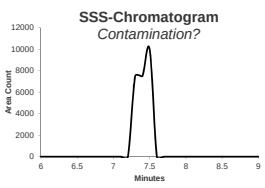
Parameter to Monitor	Source	Could Indicate
Shift in Retention Time (RT) • **gradual or sudden**	Column	degradation; not equilibrated properly; temperature change
	HPLC Buffers	preparation; evaporation; pH change; bubbles; temperature
	HPLC Pumps	performance flow rate; mixing; leaks; bubbles; change in length of tubing



SSS- Peak Shape and Additional Peaks

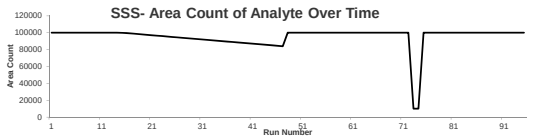
Parameter to Monitor	Source	Could Indicate
Peak Shape • Tailing, Fronting, Asymmetry	Column	degradation, not equilibrated properly, carry over
Additional Peaks	Column	carry over from previous runs/experiments
	HPLC Buffers	contamination
	Mass Spectrometer	curtain plate needs to be cleaned





SSS- Change in Intensity and Ratio

Parameter to Monitor	Source	Could Indicate
Drop in peak intensity • **Overall (gradual/sudden)** • **Increase in background noise**	Mass spectrometer	front end of MS needs to be cleaned; probe height; generator filter
	HPLC Buffers	evaporation; pH change which could affect ionization; poor quality
	Injector	partial injection; needle depth; clogged injector or loop
	Column	degradation
Change in Analyte: IS Ratio	Mass spectrometer	calibration/tuning of MS needed; charging issue



Parameters to monitor

Parameter	Tool
Instrument Performance	System Suitability Sample (SSS)
Sample Analysis Performance	**Quality Control Materials (QC) and additional parameters**
Assay Performance Over Time	Proficiency Testing (PT), Accuracy Assessments, Lot to Lot, and Instrument Comparisons

QCs Run Evaluation: Basic parameters to monitor

Parameter to Monitor	Monitor in real time and over time
Precision	Acceptable criteria set during validation
Concentration Trend	
Peak Characteristics- shape, RT, intensity, ratio	

QC Materials- Sample Characteristics

Characteristics	Requirement	Application
Type	Matrix match	MUST undergo ALL aspects of sample preparation
Number of Levels	Minimum 2 (CLIA) Recommend 3+	Concentrations- near medical decision points, over range of method
Replicates in a run	5% of total patient sample analysis	120 sample batch= 6 QCs (3 levels in replicate)
Placement in a run	Optimized to detect shifts or errors	Beginning, middle, and end
Acceptability	Established during validation *(even for commercial QC materials)*	Use trend rules and multi-rule, not just +/-SD

QC consideration for multiplexed assays and multiple instruments for a validated method

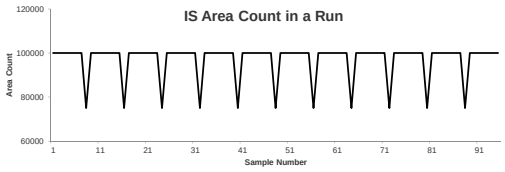
Instrumentation	Analytes	Requirements
1 System • 1 Injector • 1 Column • 1 MS	Multi	• 1 set of QCs that contains all analytes • During review analytes treated as separate runs
Multiple Systems (even same vendor/model)	Single or Multi	• QCs and Calibrators (CC) shared for method • Own set of QCs and CC for each run • Scheduled comparisons
Multiplex Approach 1 • 2+ Injectors • 2+ Columns • 1 MS	Single or Multi	• QCs and CC shared for the method • Own set of QCs and CC for each run • Schedule comparisons
Multiplex Approach 2 • 1 injector • 2+ Columns • 1 MS	Single or Multi	• QCs and CC shared for method • Split QCs and CC for each run • 1 level QC each column

Other tools: Basic parameters to monitor

Parameter to Monitor	
IS Area Count Stability	Monitor in a run and over time
Blanks	Acceptable criteria set during validation
Calibration Curve • **Slope** • **R^2** • **Area counts between runs**	Evaluate trends within and between runs
QI/CI Ratio	
Peak Characteristics- **shape, RT, intensity, ratio**	

Other tools: IS Area Count Stability

Parameter to Monitor	Could Indicate
IS Area Count Stability	Deterioration of IS Contamination Pipetting Error Sample preparation recovery Loss of detector sensitivity Ion suppression Charging



Other tools: Blanks and Calibration Curve Parameters

Parameter to Monitor	Could Indicate
Blanks • **Double Blanks** (extracted sample no IS) • **Blank** (extracted sample with IS only) • **Solvent Blank** (solvent only- no sample prep)	Interference introduced during sample preparation Carryover Degradation/Contamination of IS
Calibration Curve • **Slope** • **R^2** • **Area counts between runs**	Deterioration of calibrators Contamination Loss of detector sensitivity

Other tools: QI/CI of Samples to Report

Parameter to Monitor	Could Indicate
QI/CI Ratio	Interference

- Monitor 2 transitions for your analyte and IS
  - QI- quantitation Ion (used to determine your concentration; most abundant)
  - CI- confirmation ion
- Should not change by +/- 20 – 30% from that of the mean ratio of the standards
- Monitored within and across all samples and compared to criteria established during validation

If a run or sample is out of control, now what?

- Investigate
  - Use the whole picture
    - SSS, QCs, CC, IS, Blanks, Samples
  - Individual Sample failed
    - Can you repeat it? Is it worthwhile?
- Document all corrective actions
- Track parameters over time to monitor the performance of you method
  - catch issues before they become problems

Determine if reporting is acceptable

- **Examples**
  - Shift in RT of both IS and analyte throughout run in SSS, CC, QC, Samples- *data reportable*
  - 2 of the 3 QCs for run out of control- *data NOT reportable*
  - QI/CI ratio for individual sample outside specs- reviewed peak it was an integration problem- corrected- *data reportable*
  - Low QC fails- determined it was a loss in sensitivity, cleaned front end of the instrument check performance again with SSS- reinjection of run- run meets all criteria- *data reportable*

Parameters to monitor

Parameter	**Tools**
Instrument Performance	System Suitability Sample (SSS)
Sample Analysis Performance	Quality Control Materials (QC) and additional parameters
Assay Performance Over Time	**Proficiency Testing (PT), Accuracy Assessments, Lot to Lot, and Instrument Comparisons**

Assay Performance Over Time- Accuracy

Accuracy assessment strongly recommended – **every 6 months** or when new lot of calibrators are implemented.

Evaluation Tiered Approach (same as during validation)

1) *Best Practice*- Method Comparison to a JCTLM acknowledged Reference Method
   - JCTLM database www.bipm.org/jctlm
   - Recommend EP9 A2 40 samples
2) Evaluation of available JCTLM acknowledged reference materials
   - limited concentrations available, typically 1-4 levels
   - material should be commutable
3) Spike and recovery analysis
   - native matrix, minimum 3 concentration 5 replicates

Assay Performance Over Time- Accuracy

Consider standardization and accuracy based PT programs to evaluate accuracy

- Tier 1 Accuracy Evaluation with 40 sample comparison
  - CDC HoSt- Testosterone, Estradiol
  - CDC VDSCP- Vitamin D
  - CDC CRLMN- Lipids
  - NGSP- hemoglobin A1c
- Tier 2 Accuracy Evaluation with limited number of samples
  - NIST reference standards and other CRM
  - Accuracy Based PT Programs- CAP, DEQAS
  - NIST QAP- Vitamin D
  - CDC LSP- Lipid Standardization Program

Assay Performance Over Time- Peer Group PT Programs

- Examples- CAP, NYDOH
- Provided matrix "like" material to evaluate performance 2-3 times a year, ~5 concentration levels. Tend to be multi-analyte panels of material
- As a participate you are asked to measure this material like a real sample
- Performance evaluation based on peer group performance
- Criteria +/- 3 SD of all method mean or peer group mean
- Monitor for trends/shifts (i.e. are results always on one side of the mean?) and use CRM to investigate further CLSI GP27

Assay Performance Over Time- Accuracy Based PT Programs

- Material used in testing needs to be commutable
- No Peer Groups
- All methods evaluated against each other OR to a true value (typically assigned by JCTLM acknowledged method)
- If reference method is available evaluation can be based on accuracy
- Results can be used as accuracy monitoring as well as monitoring trends/shifts as with peer group PT

Assay Performance Over Time- Instrument Comparison

- Need with the use of multiple LC-MS/MS Systems (same/different manufactures)
- Requires instrument comparison every 6 months to evaluate performance
- Recommend following CLSI EP9A2- method comparison 40 samples
- Performance Criteria established based on performance criteria and clinical utilization of the results

Parameters to monitor

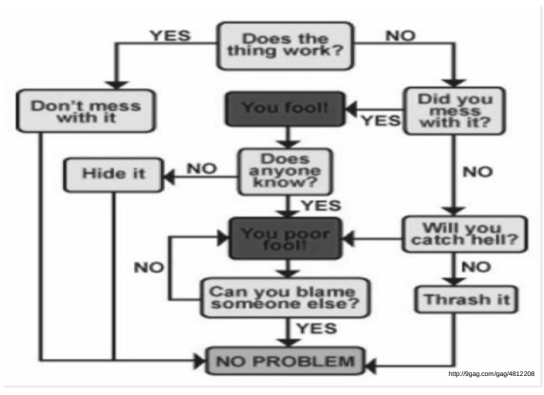
Parameter	Tools
Instrument Performance	System Suitability Sample (SSS)
Sample Analysis Performance	Quality Control Materials (QC) and additional parameters
Assay Performance Over Time	Proficiency Testing (PT), Accuracy Assessments, Lot to Lot, and Instrument Comparisons

Words to the Wise

- **Think about post implementation during development/validation**
  - What should I track? What is acceptable?
- **Use technology (excel, LIMs system etc.)**
  - Create flags (for run and samples)
  - Track long term performance
  - Constantly evaluate your parameters, are they appropriate?
  - Use calendar to schedule required post analytic monitoring
- **Train your staff**
  - What does a flag mean, why would there be a flag, is there something I can do?
- **Document, document, document**

Acknowledgement

- **C60 Document Development Committee**
  - Dr. Bill Clark
  - Dr. Ross Molinaro
  - Dr. Lorin Bachmann
- **CDC Team**
  - Dr. Hubert Vesper
  - Ashley Ribera
  - Hans Cooper, MPH



Thank You

Contact Information:
Jbotelho@cdc.gov
770-488-7391

For more information please contact Agency for Toxic Substances and Disease Registry

4770 Buford Hwy. NE, Chamblee, GA 30341
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov Web: www.atsdr.cdc.gov