# CLSI C60: Assay Validation & Post-Validation Monitoring

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EMORY HEALTHCARE
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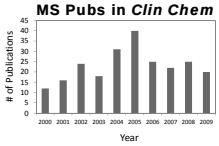
**Mass spectrometry assays:**

**Clinical**
- Testosterone, Free and Total
- Rapamycin
- Cyclosporine A
- Tacrolimus
- Busulfan
- MPA
- Vitamin D

**Translational**
- Iothalamate
- Argatroban

**Research**
- Lenalidomide
- Bile Acids
- Antidepressants
- Antipsychotics
- Plasma Metanephrines

## What you've done this far




- Determined clinical need
- Read CLSI C60
- Pre-analytical Considerations
- Internal Standard (IS) and Calibrators
- Assay Development/Optimization
- Pre Validation

## Ready to move forward…

**Pre-Validation** ✓

↓

**Validation**

- Limits of Quantitation
- Linearity and Dilution
- Imprecision
- Assay Interferences
- Accuracy

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**Post-Validation Monitoring**

- Proficiency Testing
- System Performance Monitoring

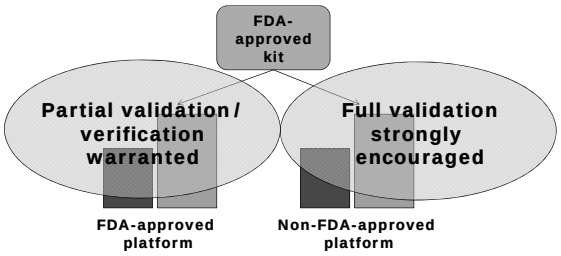
*Draft CLSI C60 still under development. Slides may not represent the final guidelines.

## Assay Validation

- Multiple analytes being measured?
  - The analytical performance of each analyte must be evaluated to ensure that the method is sufficient for use in analysis of all analytes.
- Pre-validation experiments should help
- Robustness of the method should be considered during validation
  - temperature or humidity fluctuation
  - preparation of calibrator materials by different operators
  - instrument cleanliness
  - incubation times
  - etc…
- Method re-optimization may be warranted

## But I'm using an FDA approved kit…

- It is best practice to complete a full validation of a method prior to use.




## Set Acceptance Criteria First

- Acceptance criteria must be established for each component of the assay validation prior to beginning the validation data collection.
  - Biological variation
  - Clinical guidelines established by expert groups
  - Local or regional regulatory requirements

## Limits of Detection and Quantitation

- Limit of Detection (LOD)
  - Not recommended to report values below the LOQ of the assay
- Lower Limit of Quantification (LOQ)
  - Lowest actual amount of an analyte that can be reliably detected and meets the laboratory's requirements for accuracy and precision
  - At a minimum LOQ should meet a stated acceptable precision (CV < 20%) and accuracy (< 15% bias)
  - S/N of 20:1 best practice, 10:1 minimum

## Linearity

- Linearity experiments are an essential component of testing and confirming the analytical measurable range.
  - Good to validate for each specimen matrix tested for a given analyte
  - Serial dilutions to create a linearity set should be avoided
  - CLSI document EP6

## Dilution

- Chosen diluents should be matrix-appropriate.
  - analyte-free native matrix is preferred for dilution when available.
- Dilution:
  - within the measuring range
  - outside the measuring range
  - specimens with low volume
- "Integrity of dilution" acceptability:
  - mean recovery/accuracy ±15% of the nominal analyte concentration
  - imprecision ≤15%
  - avoid dilution of specimens to analyte concentrations <3x LLOQ

## Imprecision

- CLSI EP5
  - within run precision
  - between run
  - total imprecision

| | | Run 1 | | Run 2 | | |
|---|---|---|---|---|---|---|
| Day | Date | QC level 2 | QC level 2 | QC level 2 | QC level 2 | Daily Mean |
| 1 | | | | | | |

- Stable patient sample pools are preferred
- May also be purchased through a commercial source when necessary
- At a minimum, the imprecision of each concentration level should not exceed 15% CV except for the LLOQ, where <20% CV is acceptable

## Assay Interferences

- Interference testing should be relevant to the patient population that will be tested with a given assay
  - endogenous substances - highest reported clinically relevant concentration should be tested
  - exogenous substances (drugs) - concentrations 10-fold higher than the highest concentration encountered after a therapeutic dose or patient exposure should be tested
  - tube additives - concentrations 5-fold higher than the recommended concentration should be tested
- Use ion ratio monitoring
  - qualifier ion signal >50% that of the quantifier ion, the ion ratio in the patient samples should not change by +/- 20% from that of the mean ratio of the standards

## Accuracy

- Agreement between a test result or measurement result and the true value
  - Method comparison
  - Assigned Value Materials
  - Spiking analysis
- More than one approach is recommended
- Hierarchy: authentic patient specimens

⬇

pool of patient specimens

⬇

serum-based QCs

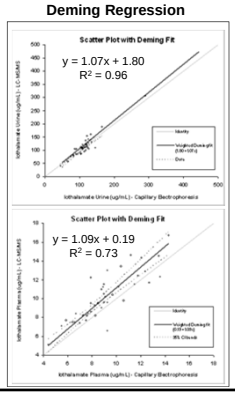
⬇

aqueous-non biological solutions

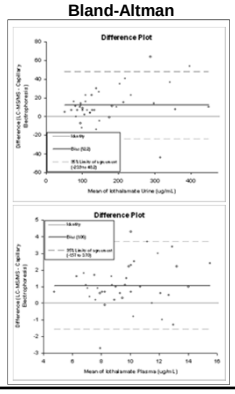
## Accuracy

**How do the methods compare?**

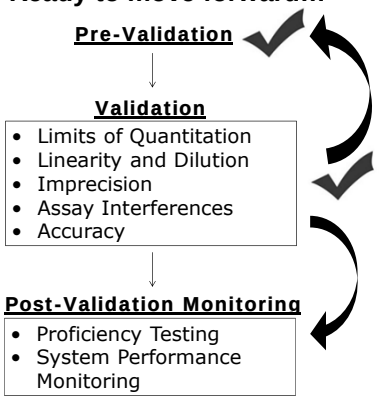
**Deming Regression**




**Bland-Altman**



## Ready to move forward…




## Post-Implementation Monitoring

- Important for:
  - regulatory compliance
  - minimization, identification and correction of analytical errors
- Scheduling of routine and comprehensive monitoring can ensure optimum LC-MS/MS system and method performance.
- Manufacturer recommendations for maintenance should be followed and incorporated into standard operating procedures.
- Frequencies depend on various testing aspects:
  - sample type (whole blood, serum, plasma, and urine)
  - sample pretreatment protocols
  - testing volumes
  - ionization sources used

## Proficiency Testing

- Methodological differences may cause systematic or random differences between results
  - differences in assay calibration material
  - MRM transitions
  - ionization conditions
  - mobile phases
  - sample pretreatment protocols
  - chromatographic conditions
- Use of certified reference materials or NIST standards (where available) may aid in the laboratory's investigation of discordant results

## Proficiency Testing

- Alternative Assessment Procedure
  - For many low volume or esoteric analytes there are no available EQA schemes in which to participate
  - In these situations a laboratory should design and implement an AAP
    - define the frequency of performance and procedures for evaluation of results.
  - Consideration should be given but not limited to:
    - the specimen source
    - the limits of acceptability
    - the range of concentrations tested
  - An external laboratory may be used as part of an AAP, provided they can measure the correct analyte in the appropriate matrix.
  - CLSI document GP29

## System Performance Monitoring

- System Suitability Samples
- Retention Time Monitoring
- Calibrator and Internal Standard Signal Monitoring
- Calibration Slope Monitoring
- Ion Ratio Monitoring

## System Performance Monitoring

### System Suitability Samples

- WHAT: non-extracted sample (ex: analyte in pure solvent) near the LLOQ is used to confirm that basic instrument performance parameters are within expectations.
- WHEN: after the instrument has equilibrated and several priming injections have been performed
- HOW: analyzed at least 3 times and the first injection values should be discarded
- Acceptability criteria:
  - basic chromatography parameters established during method validation such as the presence of correct peaks, intensity of background and analyte signal, peak resolution, retention time, and peak symmetry

## System Performance Monitoring

### Retention Time Monitoring

- WHAT: retention time for analyte and internal standard peaks should be similar to that of the standards
- WHEN: monitored both within and between runs to determine if trends are present
- HOW: for all peaks during analysis
- Acceptability criteria:
  - recommended that analyte retention times among samples should not differ by more than 2.5% for LC/MS analysis
  - a tolerance should be specified in the SOP and should be used as part of a laboratories quality assurance assessment of results

## System Performance Monitoring

### Calibrator and Internal Standard Signal Monitoring

- WHAT: peak areas for calibrators and IS should be relatively consistent within and between runs
- WHEN: monitored both within and between runs to determine if trends are present
- HOW: for all calibrator and IS peaks during analysis
- Acceptability criteria:
  - Peak areas for the internal standard in calibrators and controls should be consistent within a run, having a coefficient of variation/% relative standard deviation less than a pre-defined value for the method (< 5 - 10 %)

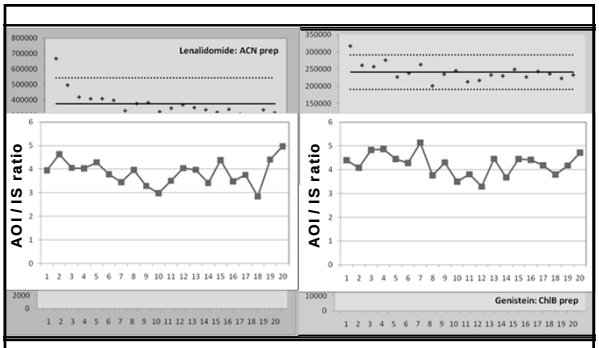
## System Performance Monitoring

### Calibration Slope Monitoring

- WHAT: should be documented and monitored for deviation from an allowable range
- WHEN: with each run
- HOW: acceptability criteria can be established by plotting all calibration data points from the method validation studies, which will provide an estimate of the variation expected for the calibration curve
- Acceptability criteria:
  - Minimal criteria include total allowable bias ≤ 15 % at all values above the lower limit of quantification, and $r2 \geq 0.995$
  - More stringent criteria may be appropriate for certain analytes

## System Performance Monitoring
### Ion Ratio Monitoring

- WHAT: ratio of T2/T1 ion
- WHEN: after patient testing
- HOW: monitored within and across all samples and compared to development criteria
- Acceptability criteria:
  - Determined during method development
  - Qualifier ion signal >50% that of the quantifier ion, the ion ratio in the patient samples should not change by +/- 20 – 30% from that of the mean ratio of the standards
  - Patient results should be flagged if the ion ratio falls out with the acceptability criteria as it may be indicative of an interference




**Checking your AOI / IS ratio over time may not detect the problem**

**Check area counts over time**

## Not addressed: Manual Reporting

**Nominal Human Error Rates For Selected Activities**

| Activity (Assume no undue time pressure or stresses) | Rate |
|---|---|
| Error of commission, e.g. misreading a label | .003 |
| Error of omission without reminders | .01 |
| Error of omission when item is embedded in a procedure | .003 |
| Simple arithmetic errors with self checking | .03 |
| Monitor or inspector fails to recognize an error | .1 |
| Personnel on different shifts fail to check the condition of hardware unless directed by a checklist | .1 |
| Error rate under very high stress when dangerous activities are occurring rapidly | .25 |

Source:Adapted from: Park K. Human error. In: Salvendy G, ed. *Handbook of human factors and ergonomics*, New York: John Wiley & Son, Inc. 1997: 163

**Report ~200 mass spectrometry results manually / day**

THANK YOU!

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