

# CLSI C60: Assay Validation & Post-Validation Monitoring

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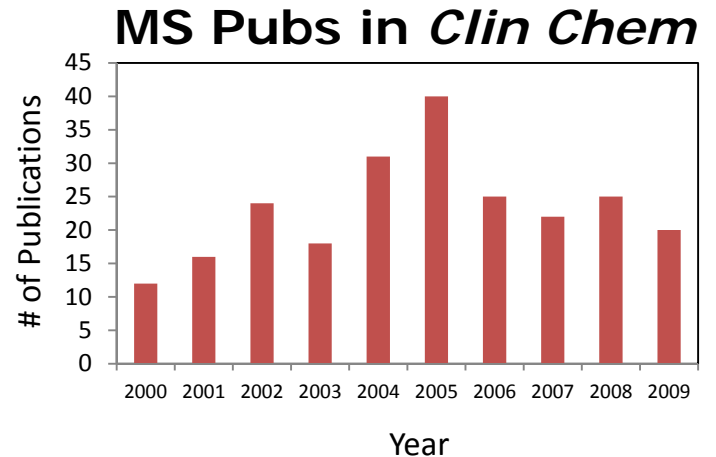


Mass spectrometry assays:

- Testosterone, Free and Total
- Rapamycin
- Cyclosporine A
- Tacrolimus **Clinical**
- Busulfan
- MPA
- Vitamin D
- Iothalamate **Translational**
- Argatroban
- Lenalidomide
- Bile Acids
- Antidepressants **Research**
- Antipsychotics
- Plasma Metanephrines

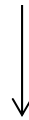
# What you've done this far

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



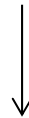
# Ready to move forward...

## Pre-Validation



## Validation

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



## Post-Validation Monitoring

- Proficiency Testing
- System Performance Monitoring

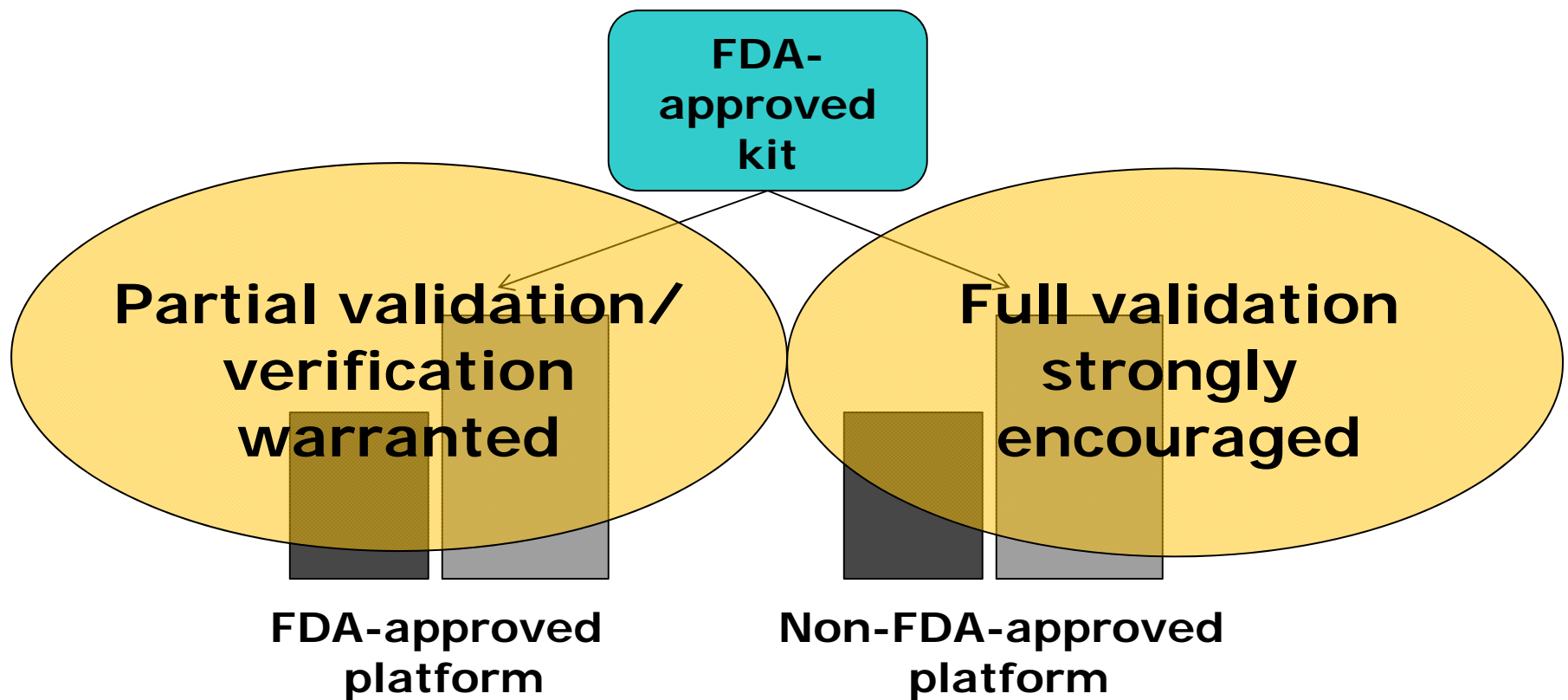
\*Draft CLSI C60 still under development. Slides may not represent the final guidelines.

# 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  $\pm 15\%$  of the nominal analyte concentration
  - imprecision  $\leq 15\%$
  - avoid dilution of specimens to analyte concentrations  $< 3 \times \text{LLOQ}$

# 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						

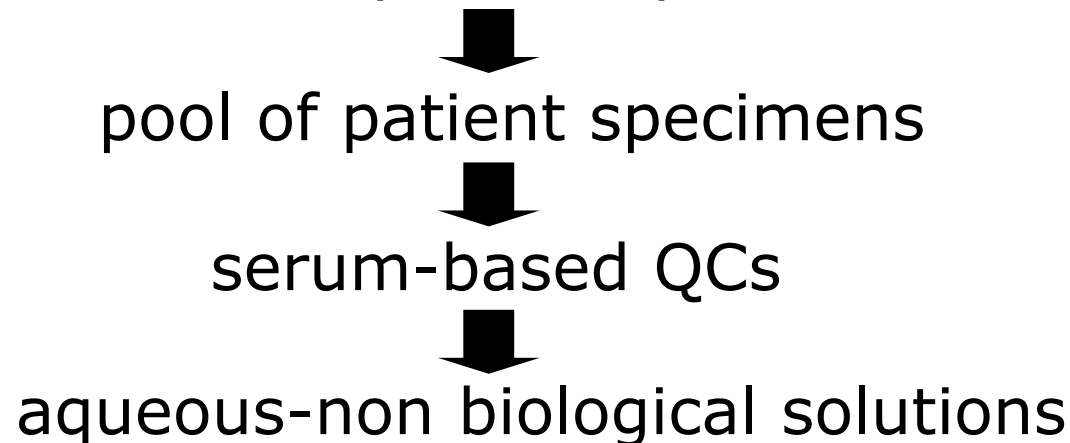
- Stable patient sample pools are preferred
- May also be purchased through a commercial source when necessary
- At a minimum, the imprecision of each concentration level should not exceed 15% CV except for the LLOQ, where <20% CV is acceptable

# 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  $\pm 20\%$  from that of the mean ratio of the standards

# Accuracy

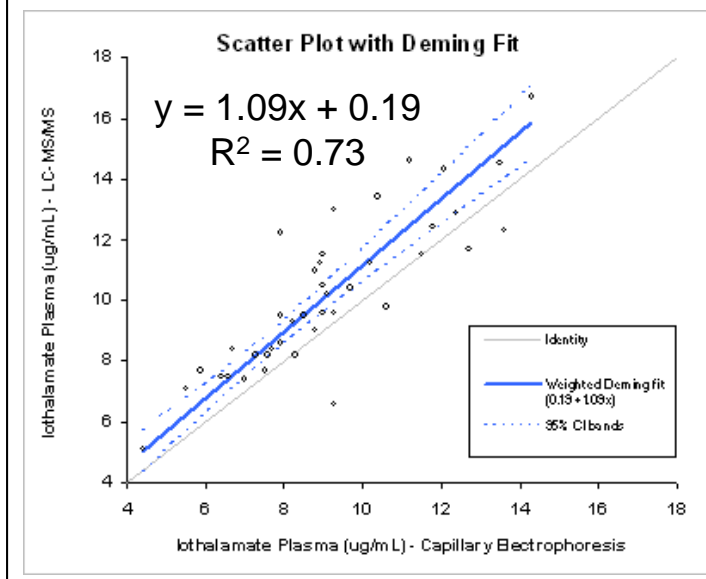
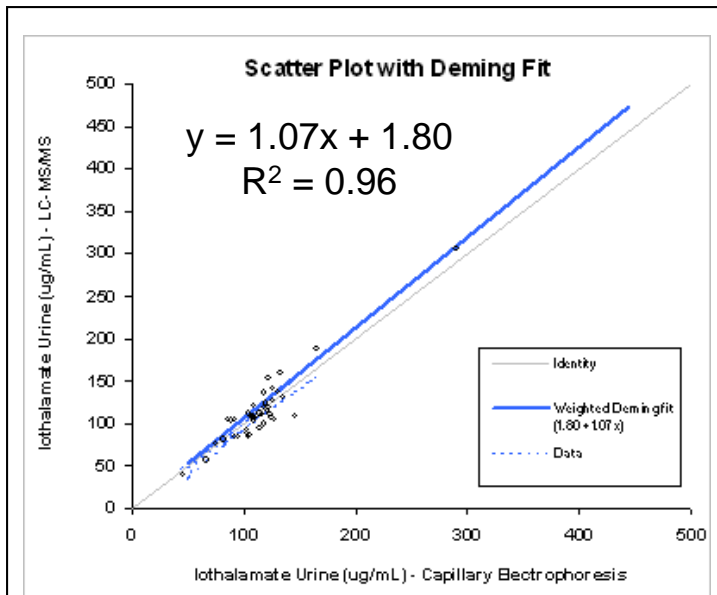
- Agreement between a test result or measurement result and the true value
  - Method comparison
  - Assigned Value Materials
  - Spiking analysis
- More than one approach is recommended
- Hierarchy: authentic patient specimens



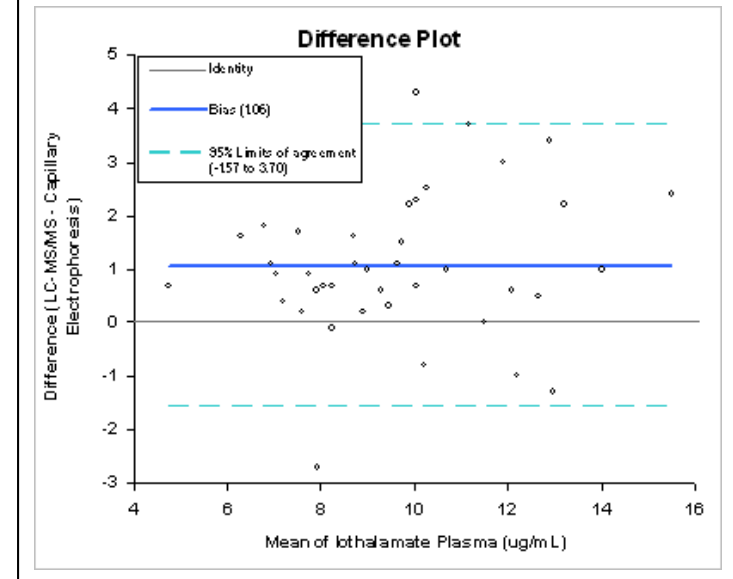
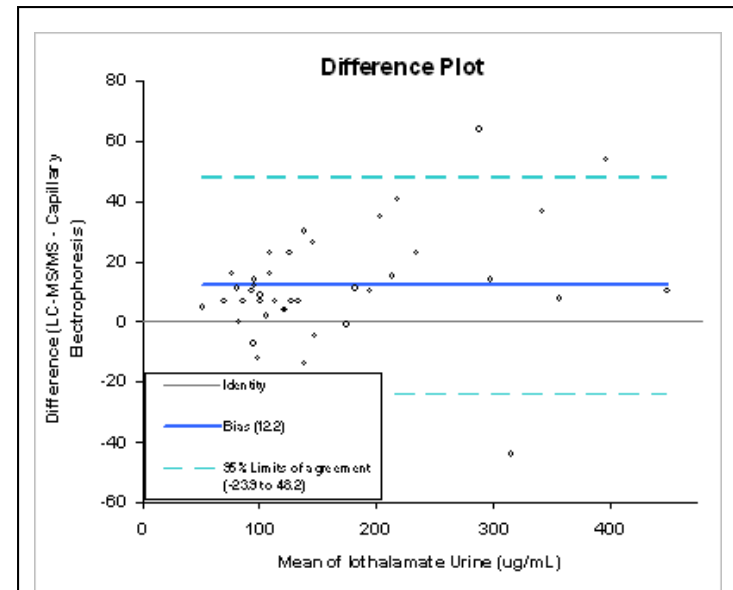
# Accuracy

How do the methods compare?

## Deming Regression



## Bland-Altman



# Ready to move forward...

## Pre-Validation



## Validation

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



## Post-Validation Monitoring

- Proficiency Testing
- System Performance Monitoring





# 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

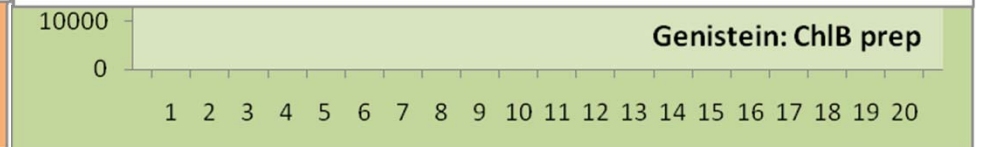
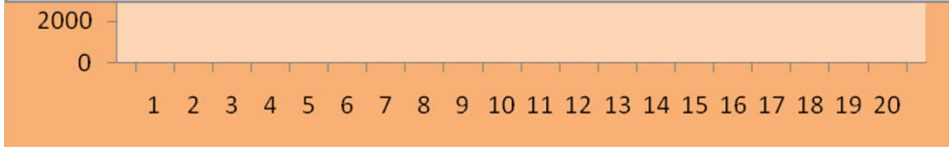
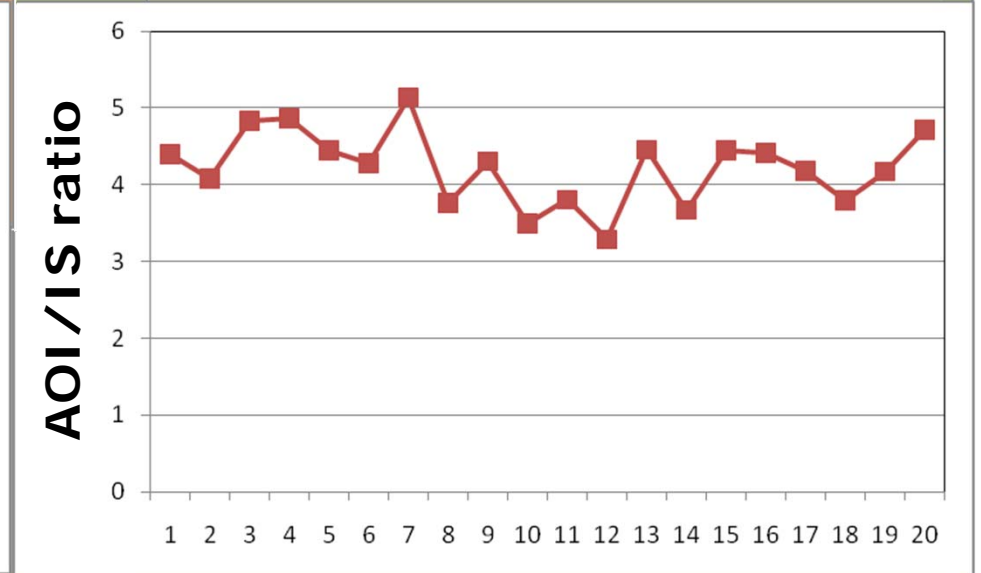
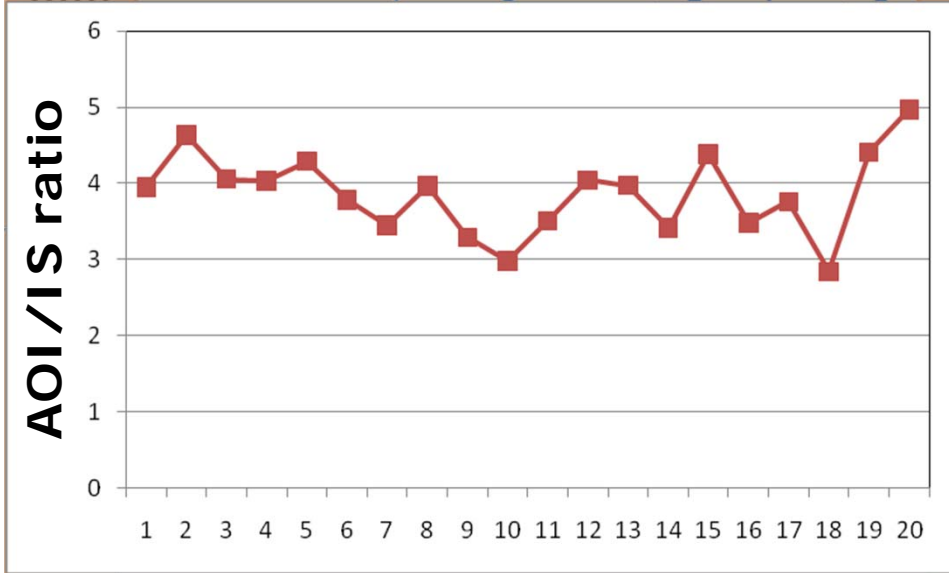
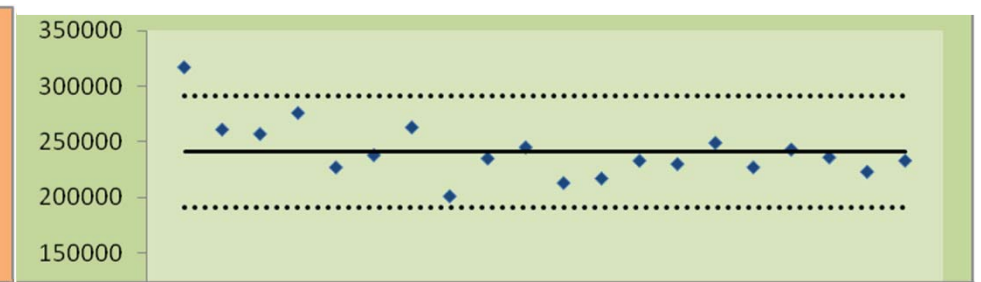
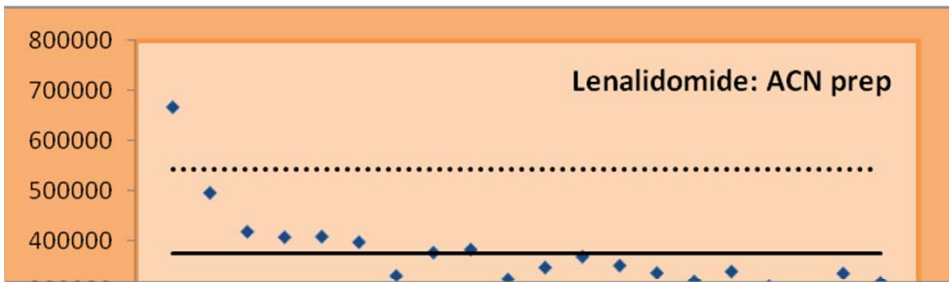
- WHAT: should be documented and monitored for deviation from an allowable range
- WHEN: with each run
- HOW: acceptability criteria can be established by plotting all calibration data points from the method validation studies, which will provide an estimate of the variation expected for the calibration curve
- Acceptability criteria:
  - Minimal criteria include total allowable bias  $\leq 15\%$  at all values above the lower limit of quantification, and  $r^2 \geq 0.995$
  - 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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