

Ready, Set, Test!

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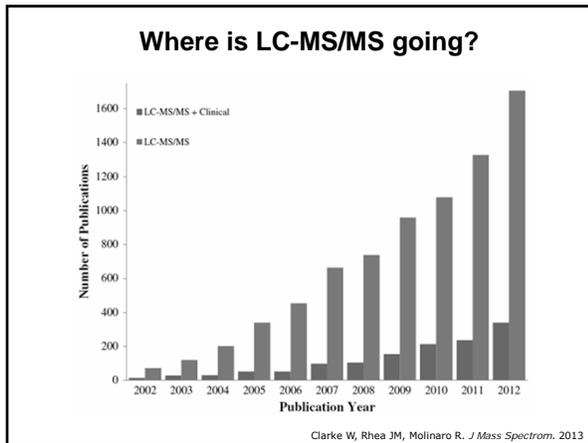


WAIT!

Financial Disclosure

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ECTRL

Emory Clinical Translational Research Laboratory

EMORY

HEALTHCARE

LC-MS/MS assays:

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

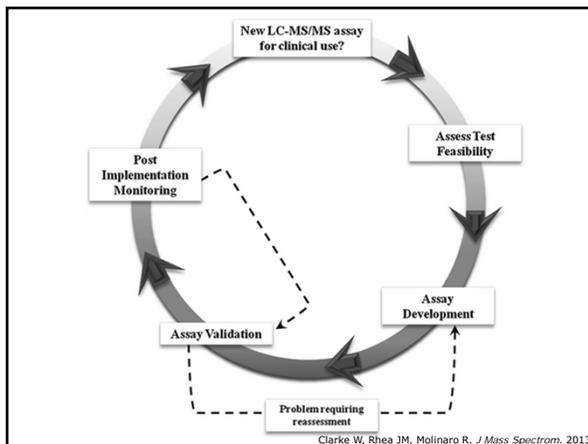
Clinical

- Iothalamate
- Argatroban

Translational

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

Research



What you've done this far

- Pre-analytic Checklist

Item	Completed
Site selection	✓
Equipment/labware selection and purchase	✓
Personnel selection and training	✓
Licensure/Compliance	✓
Hazardous waste	✓
LIMS	✓
Analyte Selection	✓
SOP	✓

Read CLSI C60 (now C62) when available

- Assay Development/Optimization

Ready to move forward...

Pre-Validation ✓

Item	Completed
Specificity and Selectivity	✓
Evaluation of Matrix Effects	✓
Carryover	✓
Simple Repeatability	✓

↓

Validation

↓

Post-Validation Monitoring

*Draft CLSI C60 (now C62) in editing phase.
Slides may not represent the final guidelines.

Ready to move forward...

Pre-Validation ✓

↓

Validation

Item	Completed
Limits of Quantitation	✓
Linearity and Dilution	✓
Imprecision	✓
Assay Interferences	✓
Accuracy	✓

↓

Post-Validation Monitoring

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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.

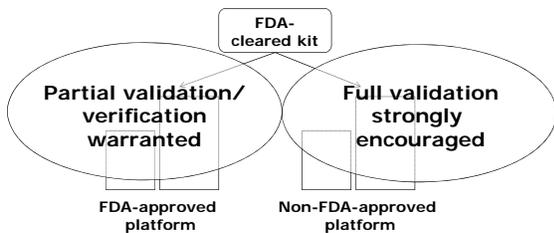
- Robustness checklist during validation

Item	Completed
temperature or humidity fluctuation	✓
preparation of calibrator materials by different operators	✓
instrument cleanliness/required maintenance	✓
stability of reagents	✓

- Method re-optimization may be warranted

But I'm using an FDA cleared 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
- 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 < 3x LLOQ (due to additive error from the dilution)

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						

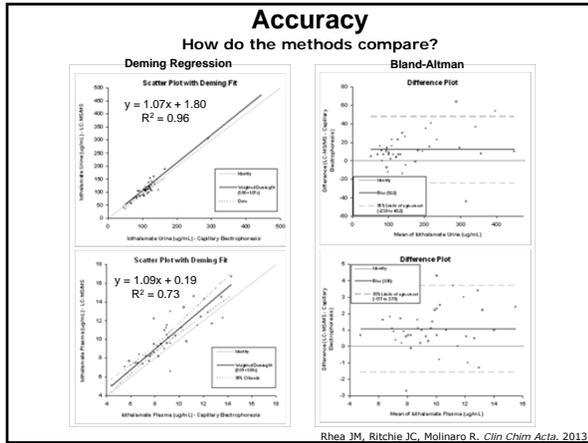
- Stable patient sample pools are preferred
- May also be purchased through a commercial source when necessary
- At a minimum, the imprecision of each concentration level should not exceed 15% CV except for the LLOQ, where <20% CV is acceptable

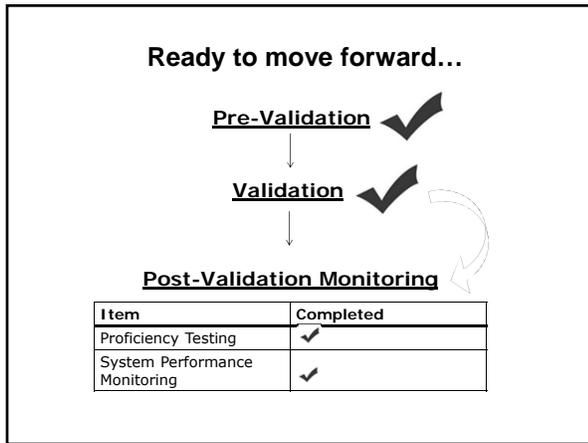
Assay Interferences

- 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/Trueness

- 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





- ### 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

Item	Completed
Retention Time Monitoring	✓
Calibration Slope Monitoring	✓
Ion Ratio Monitoring	✓

**System Performance Monitoring
Retention Time Monitoring**

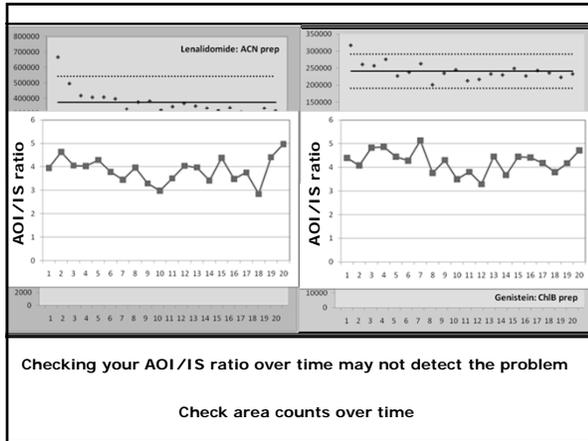
- WHAT: retention time for analyte and internal standard peaks should be similar to that of the standards
- WHEN: monitored both within and between runs to determine if trends are present
- HOW: for all peaks during analysis
- Acceptability criteria:
 - recommended that analyte retention times among 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 laboratory's quality assurance assessment of results

**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 qualifier ion/quantifier 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 $\pm 20 - 30\%$ 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



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

