

Common Mistakes in LC-MS/MS Assay Development and Deployment: What They Are and How to Avoid Them

Daniel Holmes, MD, FRCPC
Division Head, Clinical Chemistry
St. Paul's Hospital Department of Pathology and Laboratory Medicine



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Learning Objectives

- ◆ Outline traps and zaps in LC-MSMS Assay development
 - ◆ Calibrator Preparation
 - ◆ Internal Standard Concentration
 - ◆ Standing on the shoulder?
 - ◆ Sample assumptions
 - ◆ Scalability

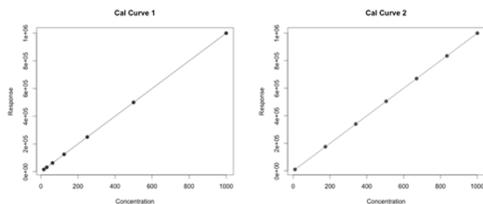
Calibrator Preparation

- ◆ To weigh or not to weigh
- ◆ What are my options?
- ◆ What are the pros and cons?
- ◆ Commercial vs In-house?

Sometimes a stone table is just not available



Calibration Concentration



Calibrators

- ◆ What should my matrix be?
- ◆ From whom do I purchase?
- ◆ How do I prove it's analyte free?
- ◆ What V:V disturbance of the matrix is permissible in spiking?

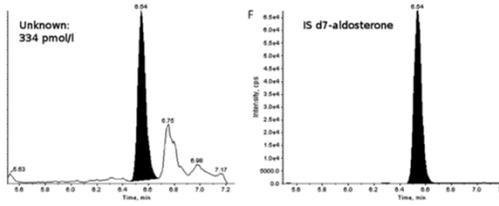
Cals: Bulk vs Daily

- ◆ Should I make up calibrators for each run?
 - ◆ Why or why not?
- ◆ What are the advantages and disadvantages of bulk calibrator preparation?
- ◆ How long will they last for?
- ◆ How do I ensure no degradation?

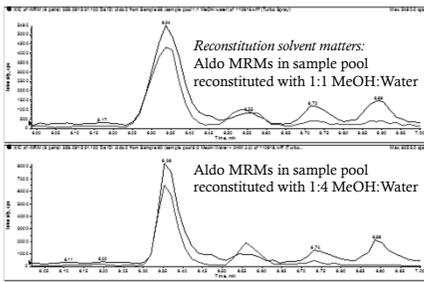
Internal Standard

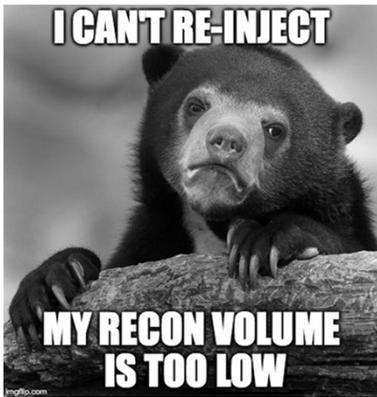
- ◆ What is the ideal internal standard concentration for your assay?
- ◆ Where should the IS concentration lie relative to the concentration of the calibrators?
 - ◆ Did you check your IS for the presence of analyte?
 - ◆ Did you check your IS for the presence of other analytes in the method?

What's wrong?



Reconstitution





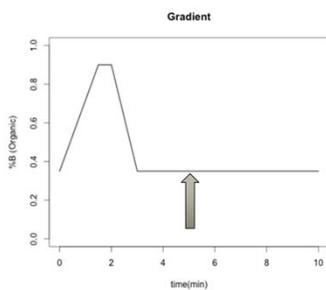
Reconstitution Volume

- ◆ Make your reconstitution volume sufficient for re-injection.
- ◆ Software crashes, PEEK tubing pops off/clogs, columns clog.
- ◆ You will want to be able to re-inject individual specimens or the whole run.

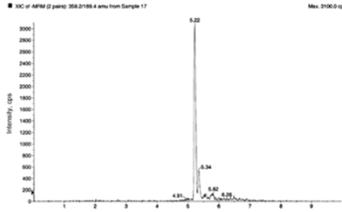
Gradient

- ◆ Standing on the shoulders of ...
- ◆ Don't assume that what is published is good and should be your starting point.
- ◆ Just because it is published, does not mean it is good and does not mean it will work optimally.

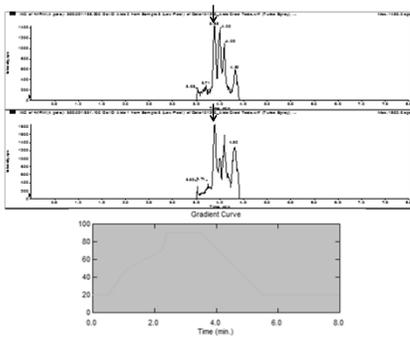
What's wrong



What's wrong?



What's wrong?



Try to see the forest...



Be suspicious

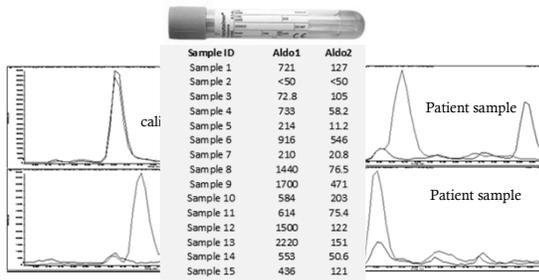
- ◆ Ammonium acetate story
- ◆ Methanol in my MTBE story

Sensitivity Wiggle Room

- ◆ Just because you have adequate sensitivity to span the clinically necessary LoQ now...
 - ◆ Columns age
 - ◆ LC-MS/MS components get dirty
- ◆ Make sure you have some room to lose some sensitivity and still be able to report the run.



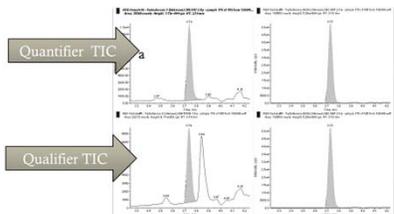
Tubes Matter



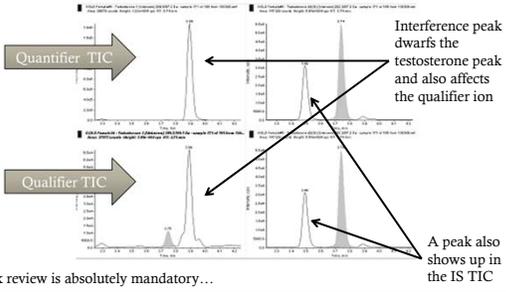
Peak Review and Peak Area Ratios are important!



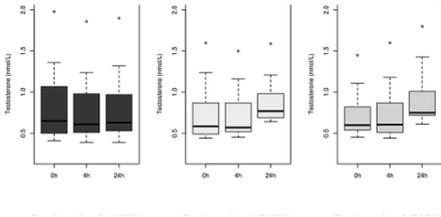
Testosterone F, Red Top Serum



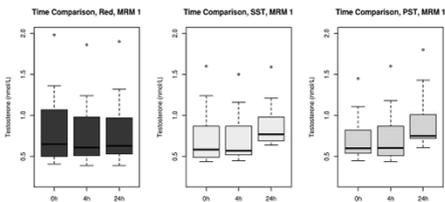
Testosterone F, Gold Top Serum



Sitting on the gel...MRM1

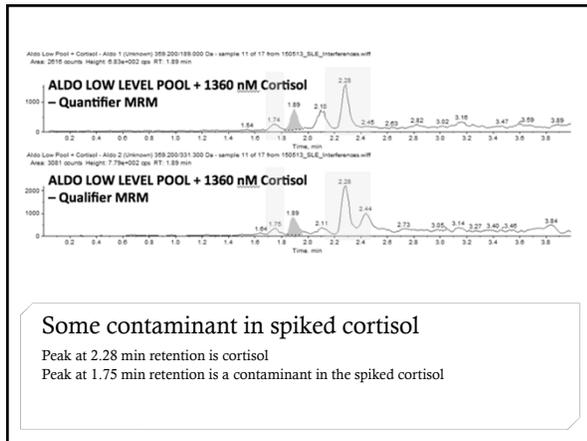


Sitting on the gel...MRM2



Interference Testing

- ◆ Spike all isobaric compounds.
- ◆ Ensure spiked compounds do not contain analyte.
- ◆ Presence of an interfering peak from spike does not mean that you have a bona fide interference.
 - ◆ Why?
- ◆ Most interferences that matter are going to be “unknown unknowns”.

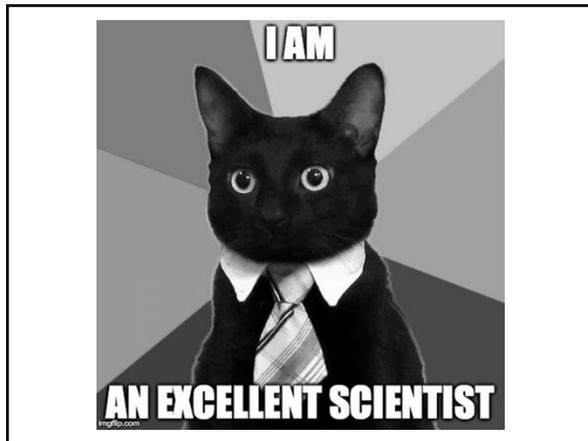


Terror on every side

- ◆ Chromatographically resolved peaks appear in the aldosterone (mw 360.44) MRM(s) when spiking
 - ◆ 18-OH-Corticosterone (362.44)
 - ◆ Cortisone (360.44)
 - ◆ Cortisol (362.46)
 - ◆ Prednisolone (360.44)
 - ◆ Corticosterone (346.46)
 - ◆ Prednisone (358.43)
 - ◆ Dexamethasone (392.46) – huh?
 - ◆ Fludrocortisone (380.45)

Stress Test Samples

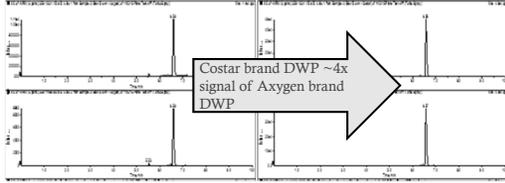
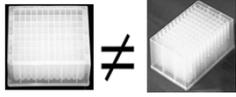
- ◆ Test samples from:
 - ◆ Patients with CKD/ Liver Disease
 - ◆ Pregnant patients
 - ◆ Pediatric patients
 - ◆ Neonatal patients
- ◆ You may find surprises



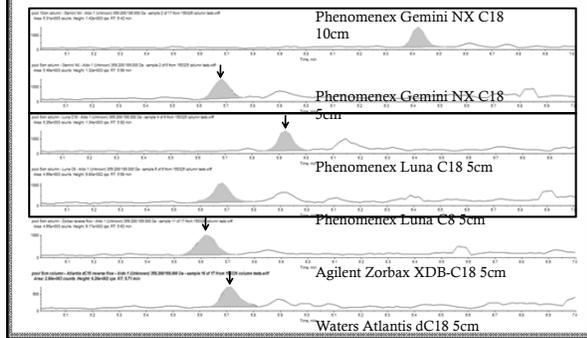
Negotiating Cluelessness

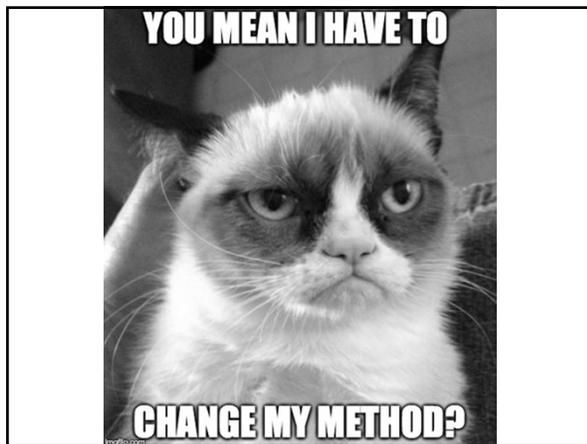
- ◆ If you are never in over your head, you are not learning to swim.
- ◆ In medicine, the dangerous trainee is not the one who lacks knowledge, the dangerous trainee is the one who is too proud to ask for help.
 - ◆ Don't bluff.
 - ◆ Do try things that you don't know how to do.

Plastic is not Plastic



Column Choice





Enthusiasm without Knowledge

- ◆ Over-commitment to the first working incarnation of your method is bad.
- ◆ Your first working method is a prototype.
- ◆ Improve it... that's actually the fun part.

Enthusiasm without Knowledge

- ◆ There are surprises ahead.
 - ◆ Clogs
 - ◆ Pressure
 - ◆ Odd samples
 - ◆ Known unknowns and unknown unknowns

Scalability

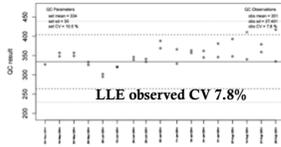
- ◆ If you build it.
 - ◆ Don't rush – can you automate?
 - ◆ But my liquid handler is so hard to program.
- ◆ I don't mind vortexing.
 - ◆ You will.
- ◆ Can I go to 96 well format?
 - ◆ Do it.

Consider Alternatives

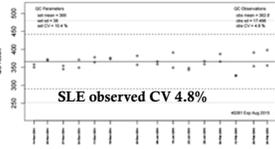


Liquid Liquid Extraction
vs. Supported Liquid
Extraction

Levy Jennings for ALDR QC Level 4 (LY1) on PAPI



Aldosterone Random (ALDR) QC LY1 on PAPI



Automate where possible

- ◆ Don't create a method that is unscalable.
- ◆ Delay in go-live to allow automation is very much worth the up front effort.
- ◆ There is no reason rush to go live only to create and unsustainable situation.



Reference Interval

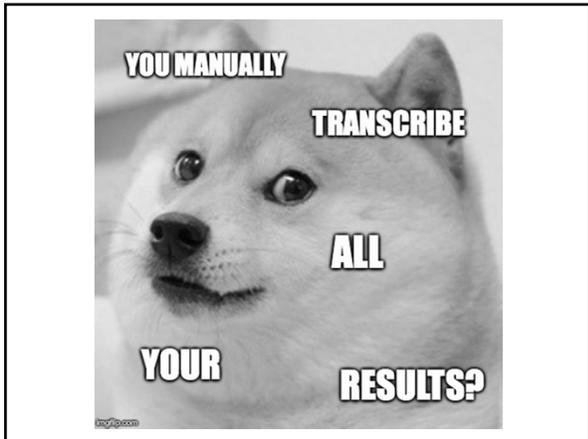
- ◆ What are your plans for the reference interval of your NIST traceable method?
- ◆ Are you going to take the reference interval from your immunoassay?
- ◆ What are your practical options?
 - ◆ De Novo
 - ◆ Comparison and Trust
 - ◆ Literature and Validation

Reference Interval

- ◆ Your method is so good, it can measure testosterone in babies, pre-pubescent children and women.
- ◆ So... how are you going to build those age-dependent reference intervals for all those age categories?
- ◆ If you produce an amazing assay with the wrong reference interval, you may have created a worse clinical situation than you had with immunoassay.

Interpretation

- ◆ So who is going to help you make the interpretation algorithm?
 - ◆ "I have a PhD. I can figure this out."
- ◆ Experience counts for a lot. Consult.
 - ◆ Even when the person with experience does not have a PhD.
 - ◆ Consultation is not a sign of weakness. It is a sign of strength.



We can transcribe...

- ◆ LIS interfacing is generally not available from LC-MS/MS companies.
- ◆ What are you going to do to get the results into LIS?
 - ◆ Were you planning to transcribe?
- ◆ What happens three years down the line when you are doing 500 LCMSMS analyses per day?
 - ◆ Do you really want to turn your laboratory staff into transcriptionists?

What Solutions?

- ◆ There are in-house and commercial solutions to the LIS integration.
 - ◆ In house: Use scripting language (Python, R) to format LC-MS/MS flat files for upload to LIS.
 - ◆ Commercial: Purchase a dedicated middleware solution from Data Innovations or other middleware company.

Intimidated?

- ◆ You shouldn't be.
- ◆ Every methodological approach is similar.
- ◆ The process seems overwhelming at first but soon becomes familiar.
- ◆ Start with an easy method—one that there is a lot of literature experience for.

Growing

- ◆ Keep the traps described in mind as you prepare your first (or next) method for production.
- ◆ Try not to rely too heavily on consultants but know when to ask for help.
- ◆ You need to be stretched in order to learn.



Questions
