Lessons Learned from Interlaboratory Method Validation Studies

The Good, the Bad, and the Ugly

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Disclaimer

- Although some of the studies in question have been funded by the USEPA Office of Water, this presentation has not been subjected to Agency review and the views and opinions are those of the authors.
- However, we do know where the bodies are buried …

Why Do We Care about Validation?

- Various U.S. laws designed to protect human health and the environment require routine monitoring be conducted by utilities, industry, private entities, and regulatory authorities.
- Depending on the regulation, environmental monitoring may require use of specific analytical methods, and various EPA programs may “approve” or recommend those methods based on data that validate their performance in the matrices of interest.
- Such data may be generated by EPA itself, by voluntary consensus standards bodies, or private companies that have developed the methods.
- While the method validation process itself and the goals may differ with the organization involved, the most widely used approach involves multiple laboratories performing the method, and generally includes samples from several different sources.

Murphy Was An Optimist!

- Anything that can go wrong, will go wrong
- That said, there is no substitute for careful planning
- Our goal is to share our experiences so that you can either avoid some of them yourself, or at least get a good laugh at our expense.

What Is Method Validation?

- Method validation is the process of characterizing the performance of an analytical method in matrices of interest.
- Involves conducting a variety of studies to evaluate method performance under defined conditions.
- May be conducted in several stages, starting with one or more single-laboratory studies, which, if successful, are followed by a multi-laboratory, or interlaboratory study.

Method Validation (continued)

- The goal is to demonstrate that analytical results produced by the application of a particular method are *fit for an intended purpose*.
- Properly designed and successful method validation studies provide data that demonstrate the reliability of the method.
- Method validation is one of several quality system components designed to ensure the production of scientifically valid and useful measurement data.
- The intended purpose for the analysis and the use of the data need to be defined upfront

Method Validation Is Not the Same as

- Proof of concept
- Method development
- Method optimization
- All those steps come before any validation studies

First, You Need a Method

- Not an idea
- Not an SOP
- A clearly written procedure in a format appropriate for the organization sponsoring the method (e.g., one of EPA's Program Offices, ASTM, Standard Methods, etc.)
- You can't just wing it by handing out some journal article

Then You Need a Plan

- There are various approaches to validation studies. Examples include:
  - ASTM D2777 - *Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water*
  - E691 - *Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method*
  - *Protocol for Review and Validation of Alternate Test Procedures for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program*
- Whatever approach you take – **write it down!**

Single-lab vs. Multi-lab

- Depending on the complexity of the method, it may be best to start with a single-lab validation study and proceed to a multi-lab study later, after you have worked out the bugs
- Preferred approach is to not have the lab that developed the method do the single-lab study
- Simpler procedures might jump straight to a multi-lab study, but it is riskier

Samples and Matrices

- Decide upfront what types of samples and matrices are of interest
- Generally driven by regulatory requirements or programmatic needs
- Not all “water” is the same, e.g., drinking water, groundwater, and wastewater are hardly interchangeable in a study
- Reagent water is not any of those, but a reference matrix
- Likewise, soils and sediments are not interchangeable
- Real-world matrices are best, even if you have to have them spiked

Number of Labs

- ASTM D2777 now says that you need at least 6 labs with useful data, but 8 is better, and to achieve that, you need to start with 10 or more
- Are they just pessimists? Hardly!
- Analytical problems happen and not all labs can overcome them easily, or in your timeframe
- Production labs may not have the time to devote to seeing the study through
- Research labs may not understand that perfection is not the goal and timeliness matters

Volunteers vs. Paid Labs

- *"You get what you pay for"* sounds like it's true
- But it's not always that simple
- And keep in mind the other old saying *"Be careful what you wish for"*

What Data Do You Need to Collect?

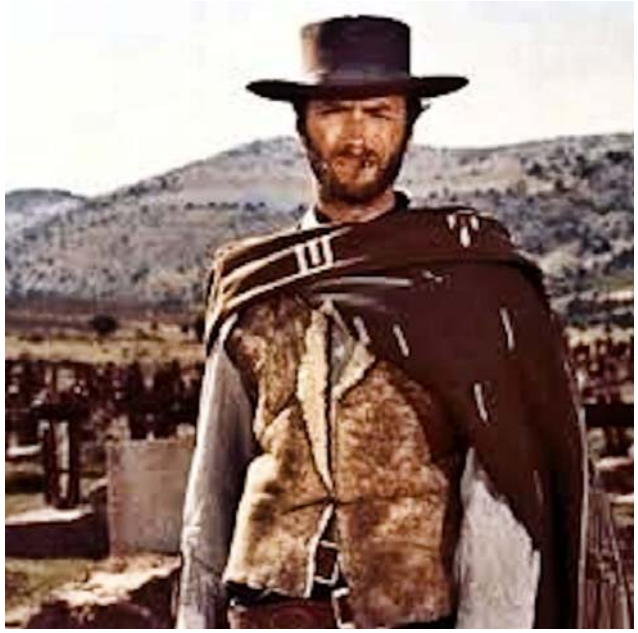
- Basically, anything and everything that the labs generate
- You may not think it's important now, but it may be too hard to get the data later when you realize that you need it
- Trust, but verify:
  - Do a formal review of all of the results
  - Spot check calculations (e.g., recalculate at least 10% of the results from the raw data)
  - Have the labs make formal corrections of any errors or discrepancies
  - Depending on the study design, there may not be firm QC criteria yet
  - Check for outliers statistically and investigate them thoroughly *before* you decide to toss them or use them anyway

How Long Is It Going To Take?

- Longer than you think
- Sometimes *way* longer … because
  - The samples could not be collected as quickly as predicted
  - The standards from the vendor had issues
  - The “clean” reference matrix wasn’t so clean
  - The government shut down
  - There was a virus going ‘round
- Whatever your time estimate is, double it. Then consider going up to the next unit of time (e.g., 2 weeks becomes 4 months)

The Good

- PAA study
- Male-specific coliphage
- qPCR for enterococci
- Method 1631



Peracetic Acid Study

- Conducted by CHEMetrics, in collaboration with EPA, Standard Methods, Hach, and others
- PAA breaks down in water fairly quickly, so shipping samples to multiple laboratories was not practical
- The method is based on a simple colorimetric test kit that can be used in the field or a fixed lab
- So instead of sending samples to labs, the “labs” were brought to a common location for the study
- 9 analysts were a stand-in for 9 labs
- Completed the lab portion of the study in 3 days

Microbiology Studies

- Male-specific Coliphage Study
  - 17 volunteer labs
  - 3 matrices (wastewater, fresh and marine waters) + PBS reference matrix
  - EPA leveraged Method 1602 for analytics and an ultrafiltration procedure to concentrate samples
- qPCR Method for Enterococci
  - 16 volunteer labs
  - Two matrices (fresh and marine waters) + PBS reference matrix
  - 7 loaner instruments provided by the vendor to labs
- Both studies were completed successfully and the validated methods are available on EPA's web site

Method 1631 – Mercury by CVAFS

- 1997 study with 12 volunteer labs, plus paid referee lab preparing study samples
- Youden pair design using samples of:
  - Reagent water
  - Freshwater, filtered and unfiltered
  - Marine water, filtered and unfiltered
  - POTW effluents, filtered and unfiltered
- 26 samples per lab, plus 6 sets of MS/MSD
- 10 labs produced data that passed the outlier tests for the Youden pairs
- EPA proposed 1631B in May 1998, and approved the method in June 1999.

The Bad

- Method 1613 study – PCDDS/PCDFs by high-res GC/MS
  - 21 labs
  - 5 countries
  - Volunteer and paid labs
- EPA biosolids methods study
  - EPA Methods 1684, 1685, 1688, 1690 and 245.X
  - 14 labs, with 4 to 10 labs per method
  - 2 referee labs preparing samples

Method 1613

- Had to use lab-prepared samples, spiked with extracts of real-world samples sent to all participants
- Even shipping those internationally was a challenge
- 4 volunteers never turned in *any* data
- Standards allegedly misappropriated by 1 international lab for use in their own study
- OCDD calculation outlier by one lab
- Took a long time, before common use of email, so many international phone calls were involved
- But we got it done, and the method was approved in 1995

Biosolids Methods Study

- Methods for Total, Fixed, and Volatile Solids (1684), Nitrate/Nitrite-N (1685), TKN (1688), ammonia (1690), and mercury (245.X)
- Referee lab sent the wrong study samples to one of the labs, which was not discovered until too late
- Did not get data from 6 labs for some of the methods, but had plenty of data for other methods
- Unfortunately, serious EPA budget cuts in the early 2000s shut down the completion of the statistical analyses and the study report and there was never funding to go back and complete the effort
- Methods for biosolids are still needed

The Ugly

- Method 1668, PCBs as congeners, by high-res GC/MS
- 14 volunteer labs, plus 1 paid referee lab preparing study samples
- Youden pair design for wastewater, biosolids, and fish tissue, using real-world samples
- Custom standards supplied to all labs

What went wrong?

- Of 14 volunteer labs, 3 never turned in a scrap of data
- 5 labs turned in unusable data for all 3 matrices, largely due to unauthorized (and often ill-conceived) method modifications
- 6 labs that did submit usable data still had some issues, particularly with method blanks
- EPA did the best that they could with the study results and submitted the method to an external peer review before proposing it at 40 CFR Part 136
- Many negative public comments lead EPA to shelve the proposal and not approve the method
- However, independent work by many labs since then has shown that the method can work well in labs with good control of background levels

Conclusions

- You can't always get what you want
- But sometimes you get what you need

- And you gotta know when to fold 'em