Biol/Chem 4900/4912

Forensic Internship

Lecture 7

Quality Assurance/Quality Control Method Validation

Method Validation is defined as

"the confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled" ISO/IEC 17025.

Method validation is a planned set of experiments to determine performance parameters.

(Will this method give me the right answer?)

QA/QC Method Validation

When do we do method validation?

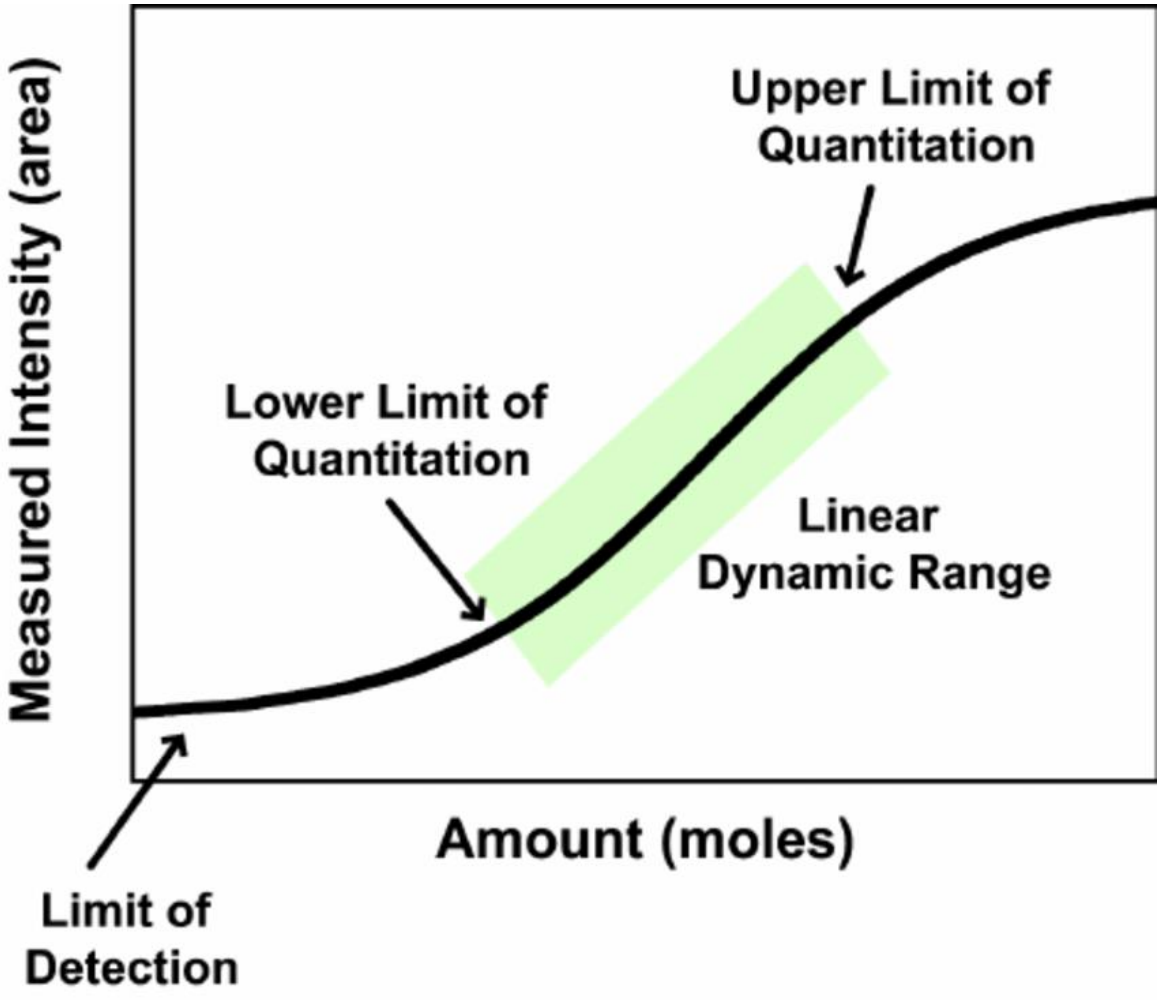
- **A new analytical method is being developed**
- **Tests are being conducted for the extension of a known method**
- **Quality control of an applied method show variability over time**
- **An analytical method has to be used in another laboratory, or with a different instrument, or by a different analyst**
- **A comparison of a new analytical method with another, know reference method is being performed**

QA/QC Method Validation

The performance parameters obtained during method validation are:

- **Selectivity**
- **Precision**
- **Bias**
- **Linear working range**
- **Limit of detection**
- **Limit of quantitation**
- **Calibration**
- **Ruggedness**

QA/QC Method Validation



QA/QC Method Validation

Ruggedness Testing

- **Evaluates how small changes in the method conditions affect the measurement results, i.e. small changes in temp, pH, flow rate, etc…**

QA/QC Method Validation

Table 2. Criteria to establish for different categories of methods of analysis [8]

Method-performance parameter	Identification test	Impurity test		Assay test
		Limit impurity test	Quantitative impurity test	
Precision	–[a]	–	+	+
Trueness	–	–[a]	+	+
Specificity	+	+	+	+
LOD	–[a]	+	–	–
LOQ	–[a]	–	+	–
Linearity	–[a]	–	+	+
Range	–[a]	–[a]	+	+
Ruggedness	+	+	+	+

[a]May be performed.

- An identification test ensures the identity of an analyte in a sample, by
- comparing it to a known RM.
- An impurity test is intended to confirm the identity of (limit impurity test) or to accurately quantify (quantitative impurity test) an impurity, defined as an entity 'which may normally not be present'.
- An assay test applies to the major component or active ingredient in a sample and quantifies the substance.

QA/QC Method Validation

Typical Order of Validation

- **Determine selectivity in the analysis of standard sample**
- **Determine linearity, LOD, LOQ, and range**
- **Determine repeatability (precision)**
- **Determine selectivity for real samples**
- **Determine accuracy/trueness based on reference material sample containing different analyte concentration levels**
- **Determine tolerance of method and interlaboratory comparisons.**

QA/QC Method Validation

Uncertainty

Uncertainty is not considered a basic validation parameter, but is usually presented in the final validation report.

QA/QC Method Validation

Interlaboratory Studies

Laboratories need to obtain an independent check of their performance.

Two main types of studies:

- **Proficiency testing schemes**
- **Collaborative studies**

QA/QC Method Validation

Proficiency testing schemes

Provides a laboratory with an independent assessment of their performance.

The assessment is expressed in terms of a score with statistics.

QA/QC Method Validation

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QA/QC Method Validation

Proficiency testing schemes

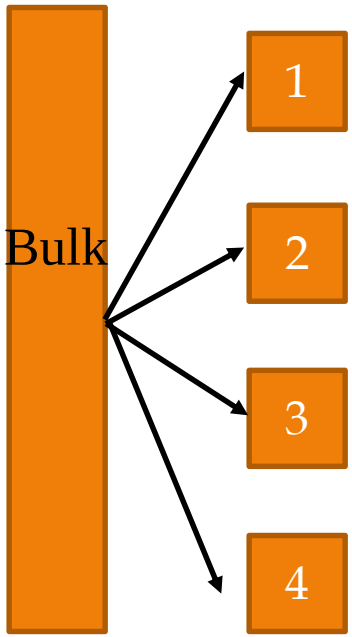
The main types of testing include:

- **Randomly selected subsamples from a bulk homogenous supply distributed at the same time to participating labs (most common – single sample distribution)**
- **Samples are divided into two or more parts, and each lab tests a subsample of each part. (split sample testing). A good way to evaluate participating labs as a potential analytical service. Can be public or closed (the lab does not know it is being tested).**
- **Sample is circulated from one lab to the next and tested. (measurement comparison scheme)**

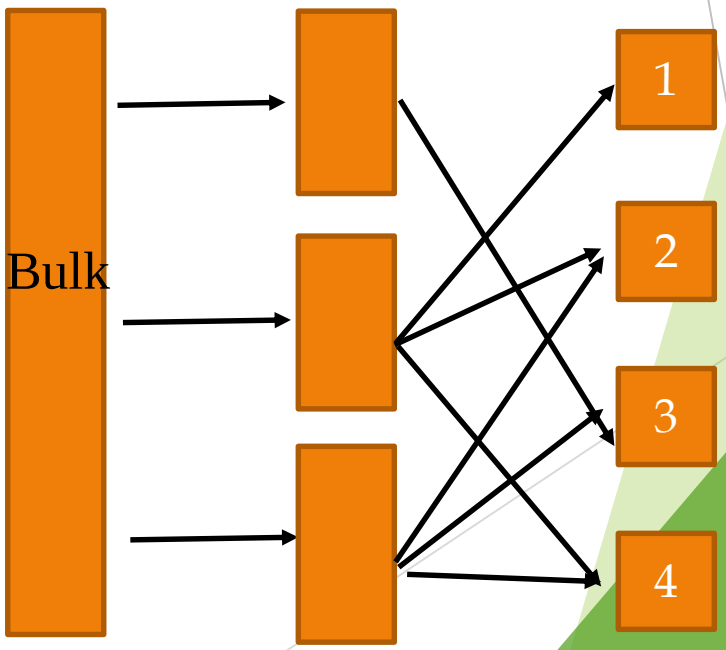
QA/QC Method Validation

Proficiency testing schemes

Single sample distribution



Split sample distribution



QA/QC Method Validation

Proficiency testing schemes

Performance values are given to the participating labs.

Most common used system is the z-score, calculated by:

$$z = \frac{(x - X)}{\hat{\sigma}}$$

where, x is the result submitted by the participating lab,

X is the assigned value, and σ is the target range.

z-score is based on a normal distribution, where 95% of the values lie within ±2 standard deviation. A score of $|z| \leq 2$ is satisfactory.

QA/QC Method Validation

Proficiency testing schemes

The z-score does not take into account uncertainty.

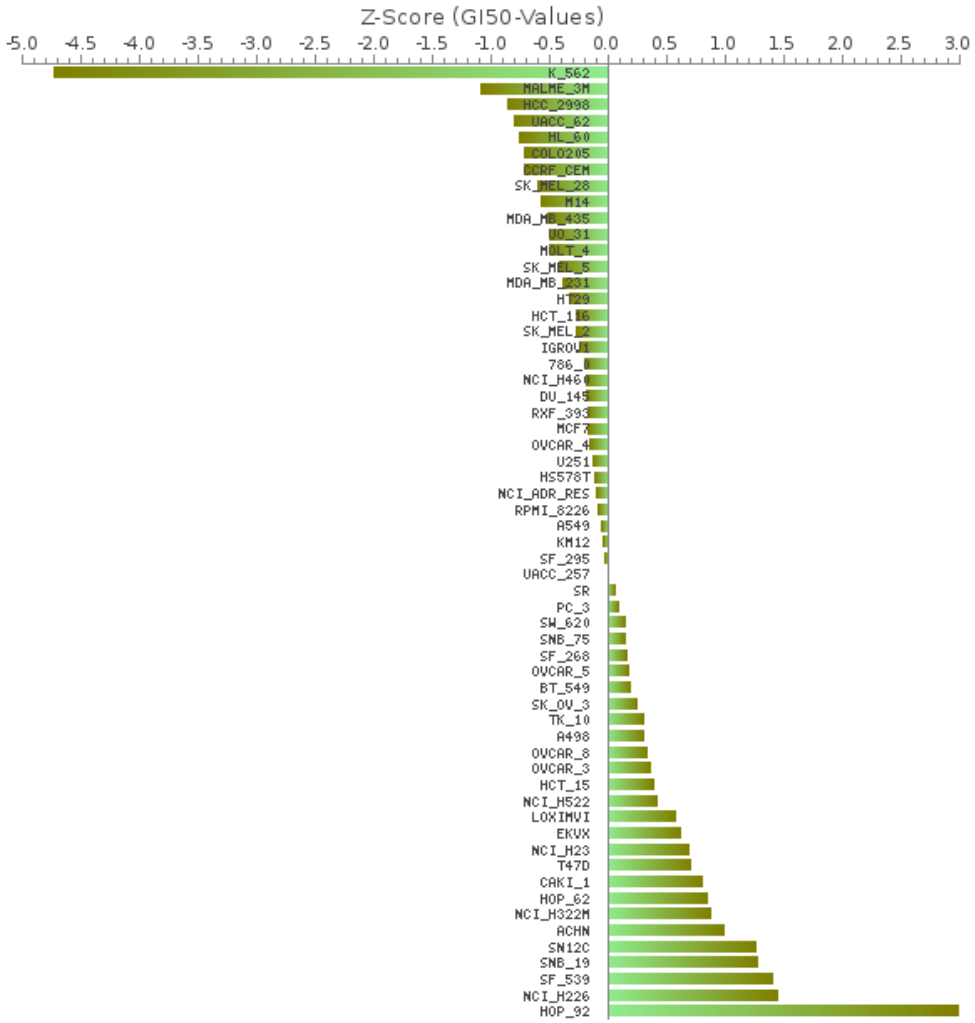
The z'-score gives uncertainty and is calculated by:

$$z' = \frac{(x - X)}{\sqrt{\hat{\sigma}^2 + u_X^2}}$$

where μ is the uncertainty in the assigned value.

QA/QC Method Validation

z-score plot for participating labs



QA/QC Method Validation

Documentation

The top-level document in a management system is called a Quality Manual. This is a generic document.

The supporting documents are Quality Procedures (central) and Standard Operating Procedures (SOPs) or Work Instructions (WIs) (local).

QA/QC Method Validation

Standard Operating Procedures (SOPs)

X-Ray Powder Instrument Operating Instructions **Chemistry**

INSTRUCTIONS FOR OPERATION OF DEPARTMENTAL DIFFRACTROMETER

CAUTION!! THE X-RAY BEAM PRODUCED BY THIS MACHINE IS EXTREMELY HAZARDOUS THE GENERATOR IS CAPABLE OF PRODUCING 60,000 VOLTS. THE ALIGNMENT OF THE MACHINE IS VERY PRECISE. THEREFORE,

DO NOT TOUCH OR TURN ANY KNOB OR PART OF THE EOUIPMENT UNLESS DIRECTED TO DO SO, NEVER OPERATE THE MACHINE UNLESS RADIATION SHIELDS ARE IN PLACE AND CHECKED VISUALLY, READ INSTRUCTIONS ALL THE WAY THROUGH CAREFULLY, BEFORE PROCEEDING.

QA/QC Method Validation

Standard Operating Procedures (SOPs)

1. **Key and Badge/Ring**

Obtain the lockout key for the instrument from Dr. Golden or X-ray Manager. Put on your radiation ring from the drawer in room 271 (this must be worn at all times when running the instrument). Insert key and turn to on position.

2. **Sign-In**

Enter all relevant information for your experiment into the logbook located next to the computer.

3. **Water supply**

Turn on the water circulator in water closet. The water temperature should read between 62 – 67 degrees and the tank water indicator light will flash. Check that all the necessary water hand valves are in the on position (located behind instrument and next to water circulator).

Check the system for leaks.

QA/QC Method Validation

Standard Operating Procedures (SOPs)

4. Sample Loading/Warmup

Prepare your sample and load into the sample holder of the goniometer by sliding the sample holder into the open slot. Clamp the holder firmly in place by pushing the center metal post under the sample holder. Close the safety door - x-rays will not be generated if the door is opened.

Make sure the X-ray tube voltage (kV) is at 0 and the filament amperage (mA) is at 0.

Press the heating standby button (middle yellow button) on the left side of the panel. Allow 20-30 seconds for warm-up.

5. …….

Assignment

- Paper – should start finishing up paper
- Begin putting together powerpoint presentation
- Homework 7
- Homework 8 – Construct a SOP for one of the instruments or methods in your lab or instrument room. Walk someone through it and have them sign it also.
- Read:

1) QA/QC topic Ch. 1-9 of Prichard

2) QA/QC topic Ch. 8 Bayne