Executing Effective Analytical Method Validations

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Validation

- Process of proving (through scientific studies) that an analytical method is acceptable for its intended use.

Methods Validation

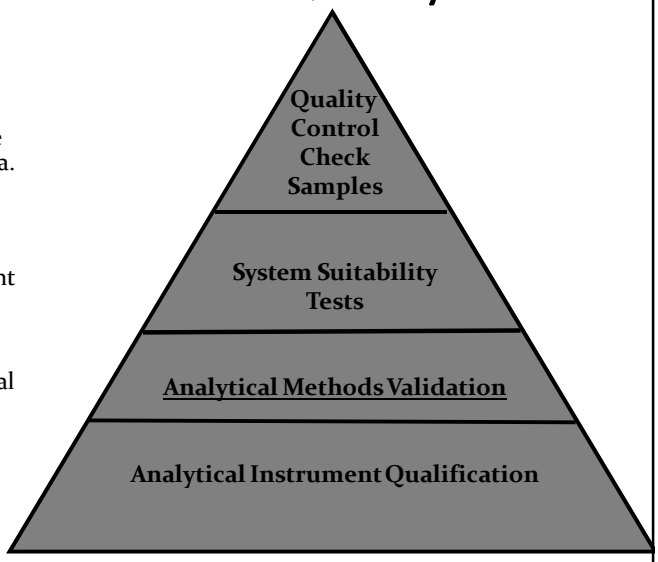
- Validation may be employed for different purposes and to different extents depending upon intent:
  - Validation of a method for compendial submission <1225>
  - Validation of a new or modified method <1225>
    - Unique to your product
    - Unique to a technology or use (e.g., cleaning validation)
  - Validation of an existing compendial method (often called verification – see <1226>

Components of Data Quality

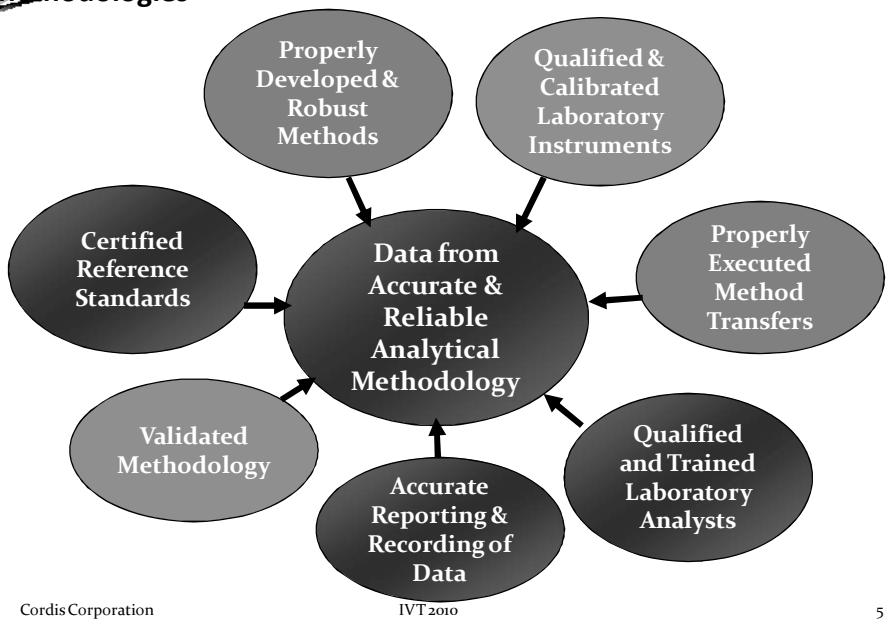
(per <1058>)

Analytical methods validation, alone, is insufficient to ensure the generation of quality data. It is one piece of the system.

Verify that the instrument qualification has been performed to meet the ranges / capabilities required by the individual assay.



Interrelated Elements Ensuring Reliability of Data from Analytical Methodologies



Goals of Test Method Validation

- Demonstration that a test method is predictable (linear, accurate, precise, specific, rugged) across the range of interest
- Demonstration that the sample preparation, instrument and method instructions are all appropriate for use and reproducible
- Goals of Test Method Transfer
  - To demonstrate that a test method can be reproduced reliably by a different laboratory on different instrumentation often with different personnel prior to first use in production

Test Method Validation

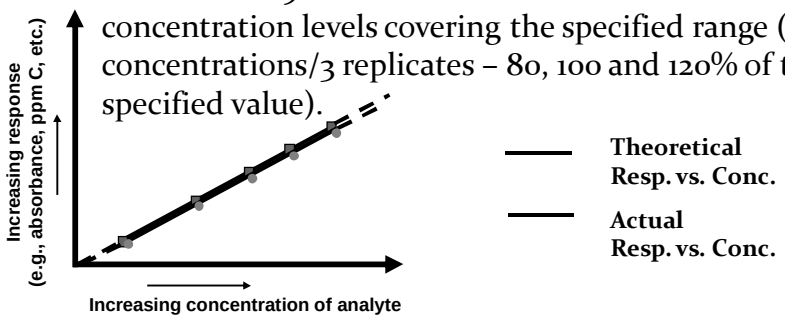
Table shows testing required by USP/ICH for all test methods by Type for the validation of a compendial method or for methods that you develop.

Assay Parameter	Category I Methods	Category II Quant.	Category II Limit Tests	Category III Methods	Category IV Methods
Assay Purpose ⇨	*Potency*	*Impurities*		*Performance (Dissolution, DR)*	*ID Tests*
Ruggedness	Yes	Yes	Yes	Yes	Yes
Robustness**	Yes	Yes	Yes	Yes	Yes

*May be required, depending on the nature of the specific test.
**Robustness should be part of development or performed here, if not

Accuracy

- Expresses the closeness of test results obtained by the method to the theoretical value.
- Recommended Data
  - It is recommended that accuracy be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations/3 replicates – 80, 100 and 120% of the specified value).



Elements of Test Method Validation (continued)

Accuracy

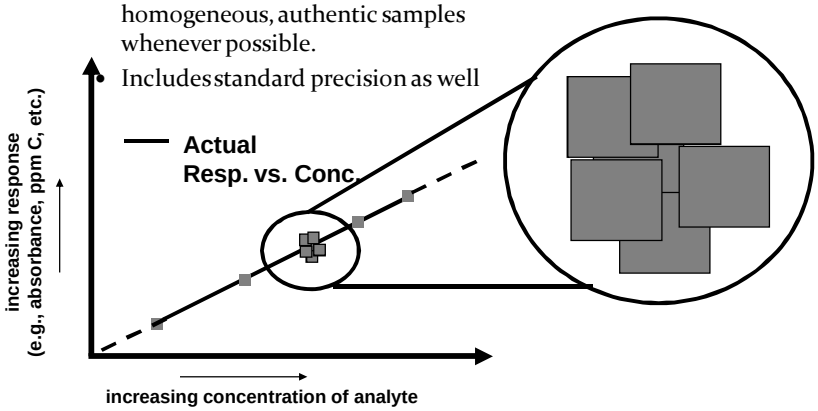
Typical acceptance criteria for accuracy include < 2% variation from the standard, although the following segregated by acceptance criteria are also possible:

Active Ingredient / Raw Materials	**98 – 102%**
Impurity / Degradation Product Biological Test	**85 – 115%**
Minor sample components (e.g., peptide counterions, preservatives, stabilizers, etc.)	**90 – 110%**

Remember to justify your acceptance criteria

Precision

- The precision of the test method expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions – base acceptance criteria on your development
- Recommended Data
  - Precision should be investigated using homogeneous, authentic samples whenever possible.
  - Includes standard precision as well



Difference Between Accuracy and Precision

Accurate, but <u>not</u> precise.

Precise, but <u>not</u> accurate.

Accurate <u>and</u> precise.

Linearity

- The linearity of an analytical test method is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
- Recommended Data
  - A minimum of 5 concentrations should be evaluated
  - Normally concentrations span range
    - ✓ 80 – 120% of the specified value for assays,
    - ✓ 70 – 130% of the specified value for DCU,
    - ✓ 50 – 120% of the specified value for impurity,
    - ✓ ±20% over specified range for Dissolution
  - Calculate <u>% recovery</u> at each point

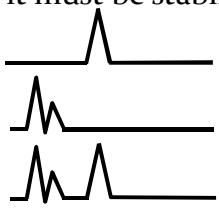
Specificity

- Ability to access unequivocally the analyte in the presence of components which may be expected to be present.
  - Must access placebo (all excipients minus the analyte).
  - Analyzing each excipient & known potential degradation product.
  - If assay will be used for stability, it must be stability indicating.

Stock Solution Active Only

Matrix, Impurities, and/or Diluent Alone

Stock Solution Plus Matrix, Impurities and/or Diluent, etc.



Adequate separation and differentiation between discrete constituents.

Stability Indicating Assay

- Assays that have been shown to be specific for an analyte, even under adverse conditions.
- A method MUST be stability indicating if it is to be used for stability testing of potency.
  - Free of interferences from excipients, analyte degradation products, excipient degradation products.
  - Able to quantitate all impurities (whether process-related or degradants).

Robustness

- A measure of an analytical procedures capacity to remain unaffected by small, but deliberate variations in method parameters.
- It should show how reliable an analysis is with respect to normal laboratory influences. These influences are tested by adding deliberate variations in the method parameters.

*** In USP 30 it was first stated that Robustness should be determined during development of the analytical procedure. This is absolutely true, but it may also be beneficial to demonstrate during your methods validation that the assay remains unaffected to those conditions demonstrated in development.**

Types of Robustness Testing

- Stability of analytical solutions
- Extraction time
- Use of filters
- For Liquid Chromatography Methods:
  - Influence of variations of pH of mobile phase
  - Influence of variations in mobile phase composition (by percentage, by manufacturer, by lot #)
  - Difference in columns (try at least 3 column lots from the same manufacturer)
  - Temperature
  - Flow Rate

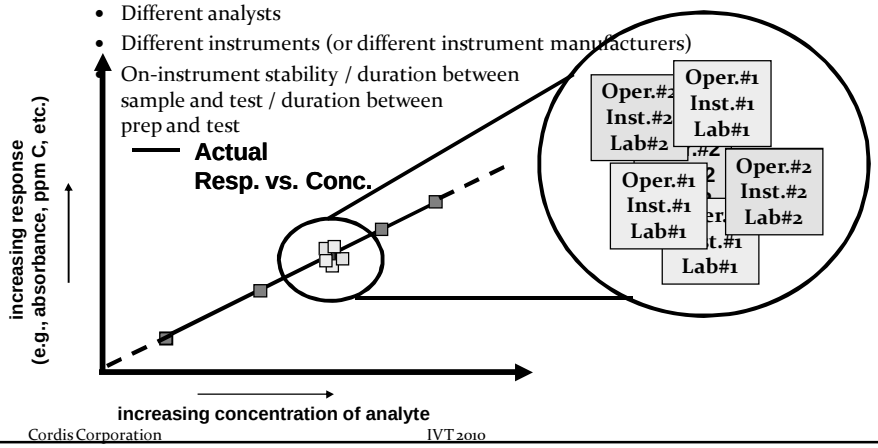
Outcome of Robustness

- All key parameters must be tested, but every step need not be robust (i.e., some steps may show <u>critical</u> influence on the assay).
  - Precautions should be added to the method indicating critical steps.
  - <u>System suitability solution and parameters should be based on the robustness testing.</u>
    - ✓ This is one reason why robustness belongs in development rather than validation. You should know your system suitability specifications prior to validation.

Ruggedness

(Intermediate Precision)

- Degree of Reproducibility of test results under a variety of <u>normal</u> test conditions, such as:
  - Different laboratories
  - Different analysts
  - Different instruments (or different instrument manufacturers)
  - On-instrument stability / duration between sample and test / duration between prep and test



LOD/LOQ

- MUST determine the limit of detection and the limit of quantitation for Related Compounds Assays (Purity Assays).
- Need to know your LOQ for limit tests.
- LOD/LOQ studies will determine accurately, at what point in your assay:
  - You can obtain quantitative results (LOQ) (typically 10:1 signal to noise)
  - The lowest limit at which you can detect (but not necessarily quantify) residue (LOD) (typically 3:1 signal to noise).

LOD / LOQ

- Signal to noise ratios may be estimated from the baseline.
- Experimental confirmation of LOD / LOQ may be obtained by injecting replicate samples at the estimated levels.
- Other approaches for the LOQ may be through the estimation of the limit from the slope of the calibration curve and the standard deviation of responses.

Potential* Elements for Compendial Method Verification

Data Elements for Verification of Compendial Methods for Dosage Forms

Technique	Cat I	Cat II Quant	Cat II Limits	Cat III	Cat IV
	Potency	*Impurities*		*Perform.*	*Identity*
HPLC / GC					
Spectrophotometric / Colorimetric					
Titrimetric					
TLC					
Gel Electrophoresis					

***Determine the requirements based upon assay criticality, complexity and your use.**

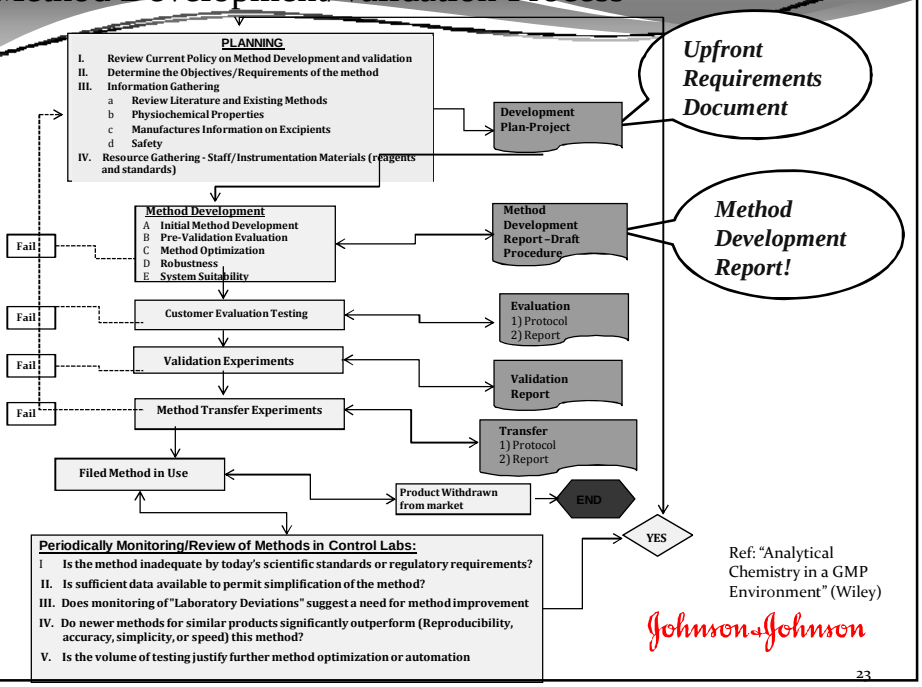
This table was present in the PF. It has since been removed for a much more abbreviated <1226>. However philosophy remains the same.

Remember ROBUSTNESS!!!!

Preventing Failures

- A robust, well-validated method will greatly reduce the need for laboratory investigations (due to invalid results).
  - Robustness studies will test the parameters of all key steps in the analysis.
  - Robustness studies will direct the incorporation of the best system suitability/control test into the analysis, through both the method and system robustness.
- Other elements that can reduce the risk of failures include:
  - Well written methods
  - Empowered analysts
  - Effective laboratory controls in all that we do!

Method Development/Validation Process



Ref: "Analytical Chemistry in a GMP Environment" (Wiley)

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Example: Practical Method Requirements

Methods Transfer

- A formal process of verifying the capability of a receiving laboratory to perform a test method, and to achieve equivalent results to the originating laboratory.
- Method transfer may be applied within a company (i.e., R&D to production QC, site to site) or between two different companies (i.e., originator to contract laboratory, or vice versa).
- In some cases, Intermediate Precision (Ruggedness) between labs during method validation can serve as the Method Transfer. This should be used cautiously. It is not meant to replace transfer to a release laboratory.

Goal of Transfer

- To ensure a clear understanding of the analytical methodology among laboratories.
- Demonstration of the receiving laboratory's ability to perform the method.
- The goal of method transfer is to determine precision (i.e., the closeness of the results between the sending and the receiving laboratory).

Prerequisites to Transfer

- Because transfer is considered a cGMP activity, all instrumentation used must be qualified.
  - ✓ These data are used as permission to proceed.
- Method must be validated, although transfer can be used to support intermediate precision.
- Transfer protocol must be reviewed and approved by all parties involved prior to initiation.

Method Transfer Flow

Initiating Laboratory	Receiving Laboratory
Deliver Docs to Receiving Lab	
What background information is provided? – Method – Validation protocol – Validation data & report – Development History	

Method Transfer Flow

Initiating Laboratory	Receiving Laboratory
Deliver Docs to Receiving Lab →	**Check Docs for Clarity – Check Instruments, Reagents, Standards**

Verify Consistency / Availability:

–Standards
- Source
- Specifications
- Storage

–Equipment requirements
- Critical control parameters & set points
- Environmental requirements
- Recommended make & model

–Reagents
- Specifications
- Supplier

Method Transfer Flow

Initiating Laboratory	Receiving Laboratory
Deliver Docs to Receiving Lab →	**Check Docs for Clarity – Check Instruments, Reagents, Standards**
Prepare Protocol	
The protocol defines formal testing and documentation to ensure the method can be run correctly at the secondary laboratory. Formal training must be performed prior to the transfer activities.	**– Protocol should include:** • Purpose • Scope • Materials • Responsibilities • Acceptance criteria

Protocol Design for Methods Transfer

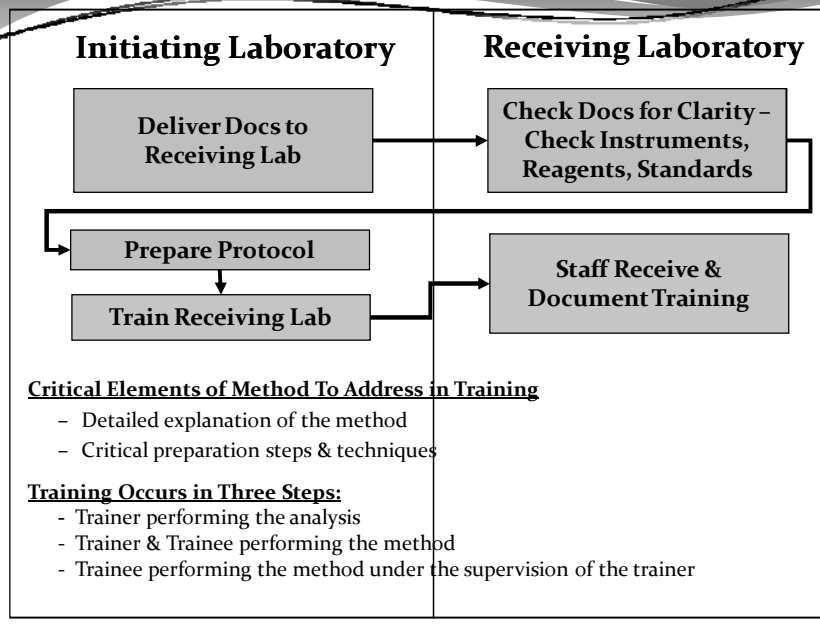
- Create formal protocols for methods transfer
- Create 2 sets of 3 – 10 samples (or spiked samples*) each representing 3 – 5 levels of analyte.
- Choose the elements to be performed, based on your method's requirements - measure *one or some* of the following on one or more instruments with a minimum of one technician from each laboratory:
  - Linearity
  - Precision
  - Accuracy
  - Quantitation and Detection limit (for impurity assays, cleaning assays, etc.)
- Values to be assessed for both within lab and between laboratory performance

*** Note: When using spiked samples either have a separate study to evaluate extraction or justify that extraction is not a critical parameter.**

Details of the Transfer

- Batches tested should be selected to challenge the method transfer.
  - Sufficient levels of impurities
  - Possibly samples spiked with impurities
  - Expired materials may be used as the purpose of the transfer is to evaluate the closeness of results between laboratories – they represent aged product and are a more realistic representation of product with impurities
- Batches should test the range of products that are likely to be encountered by the receiving laboratory.
- Receiving laboratory should demonstrate that they are able to determine the LOQ when working with low level impurities or limits tests.

Method Transfer Flow



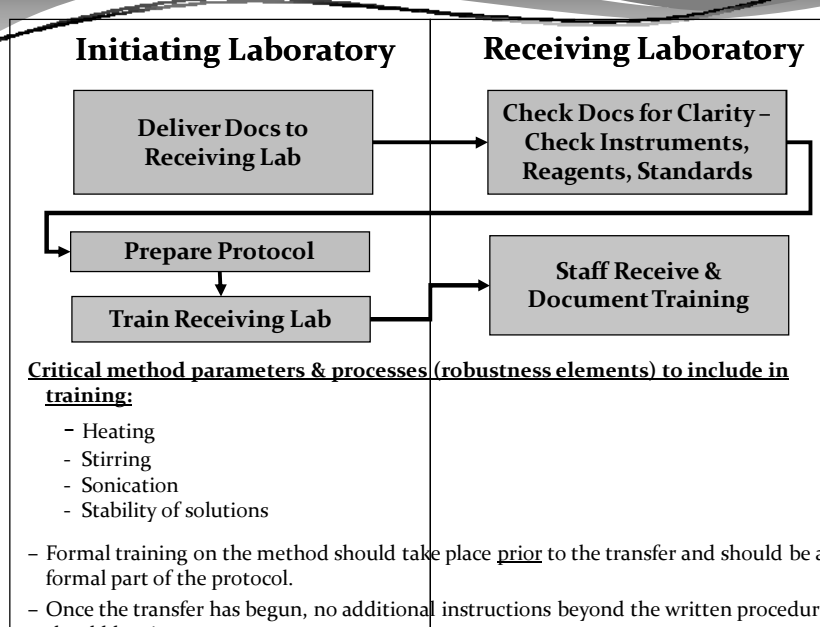
Critical Elements of Method To Address in Training

- Detailed explanation of the method
- Critical preparation steps & techniques

Training Occurs in Three Steps:

- Trainer performing the analysis
- Trainer & Trainee performing the method
- Trainee performing the method under the supervision of the trainer

Method Transfer Flow

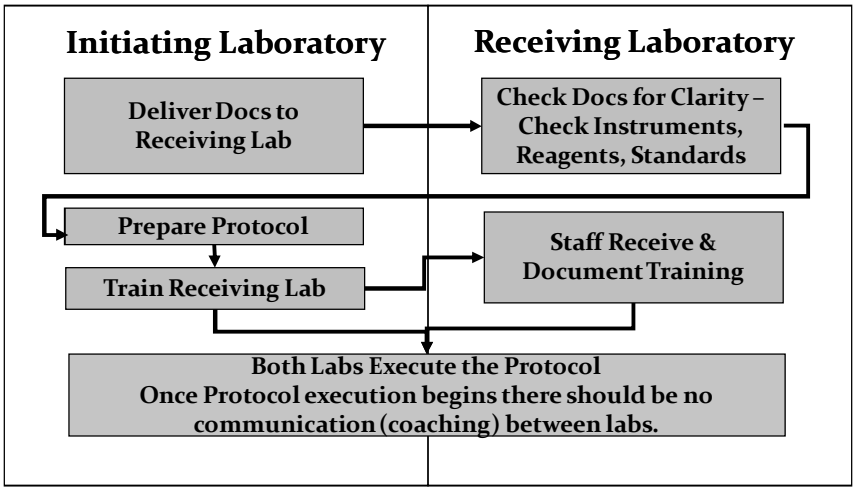


Critical method parameters & processes (robustness elements) to include in training:

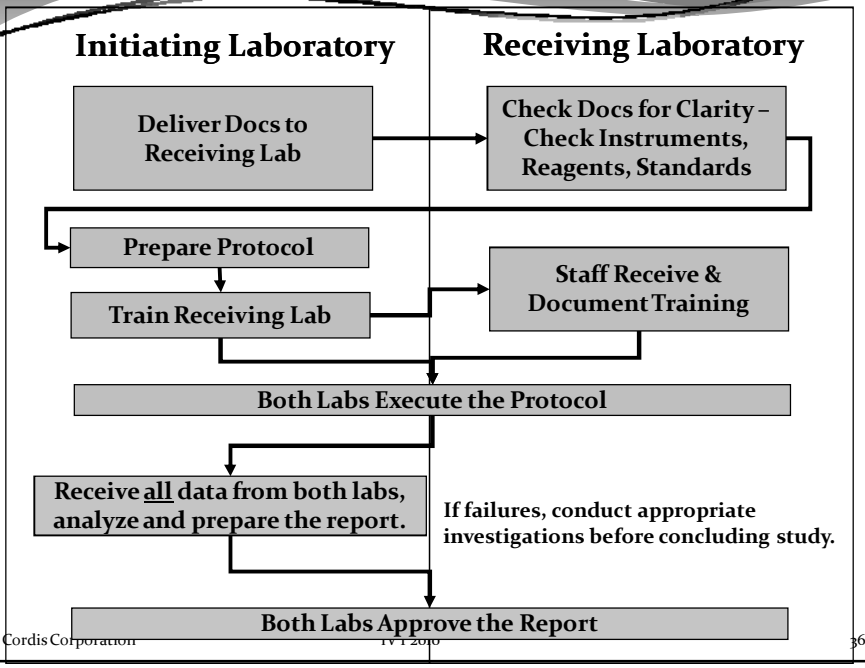
- Heating
- Stirring
- Sonication
- Stability of solutions

- Formal training on the method should take place prior to the transfer and should be a formal part of the protocol.
- Once the transfer has begun, no additional instructions beyond the written procedure should be given.

Method Transfer Flow



Method Transfer Flow



Results of Methods Transfer

- Results should be compared within analyst and between analyst for the assay
  - Individuals must meet precision of the method (typically 2 – 5% dependent on assay type)
  - Between analysts must meet the ruggedness (intermediate precision) of the method (typically 3 – 7% dependent on the assay type)
  - LOQ at the receiving laboratory must be confirmed (when required as part of the protocol)

Key to a Successful Transfer

- A well-written, robust test method
- Qualified equipment
- Well trained analysts
- Good laboratory techniques
- When methods are developed by groups other than those that will use the method day to day (i.e., R&D or contract laboratory developed methods), it is critical to ensure that the routine testing laboratory (or its designee) has input to the final procedure.

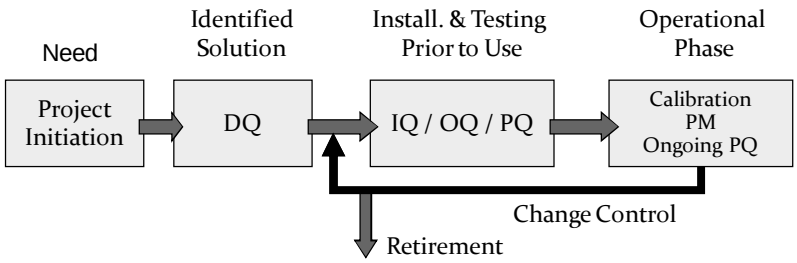
Qualification of Instrumentation

- Agency's expectation is that instrument qualification must show that the instrument is:
  - Reliable
  - Precise
  - Meets the needs of the assay
  - Functions properly for it's intended use

Regulatory Requirements

- Laboratory needs to ensure the accuracy and reliability of all data generated by their testing.
- These data are controlled through a variety of established procedures, one of which is Qualified & Calibrated Laboratory Instrumentation.

Instrumentation Life Cycle Concept



Design Qualification

- Design Qualification (DQ) represents the design or evaluation of design.
  - Defines the functional and operational specifications of the instrument and details the conscious decisions of the selection of the supplier.
  - Documented verification that the proposed design of the facilities, equipment, or systems is suitable for the intended purpose.
- Typically performed by comparing User Requirements to intended purchase / vendor qualifications / capabilities

Installation Qualification (IQ)

Typical Requirements:

- System description
- Instrument delivery
- Utility / Facility / Environment
- Assembly & Installation
- Network & Data Storage
- Installation Verification
- System Check

➢ Needed Documentation:

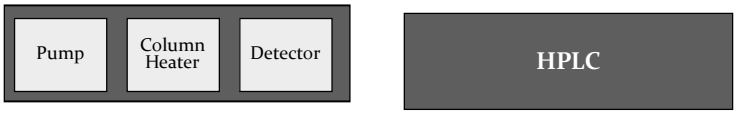
- Manuals
- Drawings
- SOPs Drafted or at Least Named
  - ✓ Setup
  - ✓ Operation
  - ✓ Cleaning / Calibration / PM
  - ✓ Data management / backup

Operational Qualification (OQ)

➢ Documented verification that the equipment when assembled and used according to SOPs does, in fact, perform its intended function.

➢ This is to ensure that the installed instrument works as specified.

➢ OQ is usually performed both modularly and holistically.



Modular Holistic

Needs for a Proper Operational Qualification (OQ)

- Knowledge of:
  - What will the instrument be used for?
  - Will all the functions of the instrument be used?
  - Over what range, of a particular parameter, will an instrument be used?
  - What tolerance limits are critical?
  - How best to test to obtain the maximum amount of information from the minimum amount of data?
  - Based on the risk assessment for the product and its testing, what frequencies / criticality should be assigned to the calibration and PM?

Operational Qualification (OQ)

Typical Requirements:

- Test of Instrument's Fixed Parameters
- Secured Data Storage, Backup and Archiving
- Instrument Function Tests (for intended use)

Needed Documentation:

- Manuals
- SOPs for Operation and Data Management
- Test scripts that truly challenge the instrument functionality based on your use

Performance Qualification (PQ)

- Process of demonstrating that an instrument consistently performs according to a specification appropriate for its specific use.
- This ensures that the system operates optimally. These tests should be performed on a complete system.
- PQ may be modular or holistic, but holistic testing is often more meaningful.

PQ Pitfalls

- Don't attempt a PQ on a poorly maintained instrument.
- Don't perform a loose PQ. Challenge the equipment at or ideally slightly beyond its true needs.
- Ensure that the PQ reflects the intended purpose of the assay and the type of analyte:
  - Method purpose – impurity, potency, functional assays
  - Sample type – drug substance, excipient, impurity, preservative

Contents of PQ Documentation

- Performance Qualification (PQ)
  - Describes testing to ensure that the instrument performs reliably and reproducibly under the firm's:
    - ✓ Laboratory conditions
    - ✓ Operating specifications
- Verify that the instrument is capable of doing the job we need it to do
  - Is unique to each product/facility
  - Preferably performed by an internal person intimately involved with the particular product/test
  - Use worst-case examples from the laboratory
  - Use existing assays in a test environment
  - Consider testing controllable causes of variation

Requalification

- Determination that an instrument, after repair, service, move or other adjustments is operating as intended
- Requalification may include the repeat of <u>all</u> or <u>some</u> of the original qualification testing depending on the change and its likely impact
- FDA expects that all instruments used for analysis are:
  - Reliable
  - Precise
  - Functioning properly for intended use

Questions?