Design of Method

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Outline of Presentation

- Role of Analytical Methods in Assuring Quality
- Selection of Analytical Methods
  - Compendia
  - Commercial Kits
  - In-house Developed
- Design of Analytical Methods
  - Reference Standards & Controls
  - Statistical Methods, Reportable Results
  - Format
  - Examples of System Suitability
- Managing Variability/Risks - Example

Analytical Methods in Assuring Quality

- Critical in Assuring Quality for both Conventional and New Paradigms
  - Drug Development
    - Discovery
    - Formulation Development
    - Pre-Clinical (Pharmacology, Toxicology, Immunogenicity)
    - Clinical (Pharmacology, Immunogenicity)
  - Validation Studies
    - Process Validation
    - Cleaning Validation
    - Viral Clearance Studies
  - Comparability Studies

Analytical Methods in Assuring Quality

- Critical in Assuring Quality for both Conventional and New Paradigms
  - Manufacturing
    - In-Process Testing and Characterization
    - Formulation
    - Monitoring (PAT) – Critical Tool of QbD
  - Release Testing
    - Raw Materials
    - Intermediates
    - Components (API), Drug Substance, Drug Product
  - Stability Testing
  - Consistency in Manufacture

Selection of Analytical Methods/Probes

- Purpose of Test (Monitoring)/Intended Use
  - To Measure Purity, Yield, Release, Stability
  - Preferably Online or Atline Monitoring or Rapid
- Sound Scientific Principles
  - Process Decisions and Quality Depend on Method
- Method Capability/Inherent Variability
  - Important for Process Decisions and in Defining Design Space
- Technology/Instrumentation
  - Compatible with Manufacturing Environment
- Big Picture Consideration – Same Technology/ Method with appropriate Design can be Used in Formulation, Pre-Clinical & Clinical Evaluation, Lot Release and Stability Testing

Characterization Tests

- Tests used Extensively during Development to Understand Product (Structure, Purity) and Mechanisms of Action (Proof of Concept)
- Scientifically Sound, Providing Reliable Results
- Usually Not Validated, Varying Degree of Qualification
- Usually Not Part of Release Tests
- Used in Support of Process or Other Changes to Approved Applications

Selection of Analytical Methods Compendia Methods

- Need an SOP or Test Method
- Full Validation Not Required
- Verification – Demonstration of Suitability Under Actual Conditions of Use
- Studies on Interference by Matrix
- Examples – Sterility, Endotoxin, Bioburden, Residual Moisture, Chemical Tests

Selection of Analytical Methods Considerations for Commercial Kits

- Need an SOP or Test Method
- Appropriate Product Related Controls
- Qualification/Validation Required, as appropriate
- Robustness – Lot to Lot Variability
- Consistent Supply of Kit

Design of Analytical Methods Critical Equipment & Reagents

- Identify Critical Equipment, Software
  - Having Direct Impact on Results
  - IQ/OQ/PQ (Usually Verified during Inspections, may be Requested for BLA, Supplements)
- Define Critical Reagents
  - Having Direct impact on Results
  - Each New Lot Qualified for Suitability
  - Examples, Cells, Antigens, Antibodies, Conjugates, Challenge Toxins, Viruses

Design of Analytical Methods Reference Standards and Controls

- Define Reference Standards & Controls
- Reference Standard Against Which Test Results are Calculated
  - Commonly Used Except for Viral Infectivity Assays
- Controls – Part of System Suitability
  - Validity of Method

Design of Analytical Methods Reference Standards

- Non-availability of Standards/Reference Preparations for New Products
- Selection of In-house Reference Preparation
  - Evolvement During Product Development
  - Discussion During Pre-IND, early IND Phases
  - Later Stage (Phase III) – Reference from Same Lot as Clinical Lot (Primary Standard)
  - Similar as Product or Different

Design of Analytical Methods In-house Reference Standards

- In-house Ref. Preparation
  - Method of Preparation and Characterization
  - Stability, Storage, Expiration Dating
  - Enough Quantity (Shared with Regulatory Agencies)
  - Calibration (Development of Primary Standard, not Serial Calibration against Last Ref.)
  - Track & Trend Parameters of Standard Curve

Design of Analytical Methods Controls

- Controls – Part of System Suitability
  - Negative, Low, Medium, High
  - Defined Parameters (95 or 99% Confidence Intervals)
  - Similar to Product, Test Samples
  - Same Issues for Selection as for Reference Preparations
  - Track and Trend (Method Performance)

Design of Analytical Methods Optimal Conditions

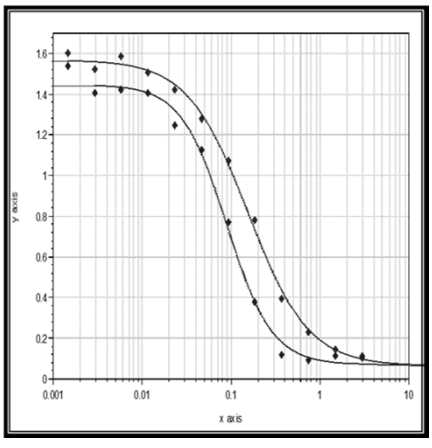
- End Point Results
  - Titers (Highly Variable, Day to Day, Lab to Lab)
  - Against Reference Standard
- Optimal Conditions
- Background (Affects Specificity/LOD/LOQ)
- Blocking Agents (Controls Background)
- Appropriate Controls (System Suitability)
- Study Robustness
  - Ranges around Temperatures, Time, pH, etc.
  - Different Lots of Critical Reagents, Columns, etc.

Design of Analytical Methods Statistical Methods

- Standard Curve
  - Linear – log, probit
  - Immunoassays - Nonlinear, Sigmoid Curve
  - Non-linear statistical models
- Calculation of Results from Standard Curve
  - NO Extrapolation
  - Parallelism is Important
  - Use Linear Part of Curve above Background

Four Parametric Logistic Equation

- Enough Points to Generate Upper and Lower Asymptotes
- Slope
- 50% End Point
- Results from Linear Part of Curve
- r^2
- Parallelism



Examples – System Suitability Physico-Chemical Methods

- Fundamental Part of procedure
- Chromatographic Procedures
  - Injection Repeatability
  - Efficiency or Resolution
  - Asymmetry (Tailing)
  - S / N for Quantitative Impurities and Limits Tests
- Non-chromatographic procedures
  - Titration - Blank
  - Polarimetry - Rotation Standards
- Trending and Tracking (During Inspections)

Examples – System Suitability Immunochemical Assays

- Standard Curve Parameters
  - Linear – r, Slope, Intercept, 50% End Point
  - 4 – Parametric, Upper & Lower Asymptotes, r, slope, 50% End Point
- Background (Affects LOD, LOQ)
- Parallelism
- Internal Controls
  - Limits Sets at 95 or 99% CI
- Trend and Track (During Inspections)
  - Reflect Method Performance Over Time

Method Design – Format

- Purpose of Test/Intended Use
  - Proof of Concept/Product Characterization
  - Manufacturing Process Validation Studies
  - Support Process Changes
  - Formulation of Bulk (Drug Substance)
  - Release Test/Stability Test
- Inherent Method Variability
- Risk (Analyst and Instrument Errors)

Method Design Risk Mitigation

- Un-Detected Mistakes (Risks)
  - People Make Mistakes
  - Unexpected Malfunction of Instrument
- Subjectivity in Observations (Risk)
  - Independent Observations by Two Analysts
- Inherent Variability of Method
  - All Methods Have Variability
  - Lesser (Physicochemical)
  - More (Biological/Animals)

All Measurements have a degree of Uncertainty

- All Methods have Inherent Variability
  - Whether Method with given Inherent Variability "Suitable for Intended Use"
  - Is there any way to Manage Inherent Variability?

All analytical measurements are wrong; it's just a matter of how large the errors are, and whether they are acceptable (Thompson, 1989, Analytical quality control in theory and practice. In Proceedings of 3rd International Symposium on Harmonization of Quality Assurance Systems in Analytical Chemistry, Washington, DC, ISO, pp. 183-189.)

Mike Thompson, Imperial College, London

Typical Intermediate Precision (CV)

Type of Method	Typical CV
Physico-Chemical, HPLC	<10%
Immuno-Chemical, SRID, ELISA	10 – 25%
Microbiological	15 – 25%
Animal Assay (Neutralization)	<10%
Animal Immunogenicity	Up to 50%
Viral Neutralization (Titers)	<25%
Viral Infectivity Titration	167%

Managing Risks and Inherent Method Variability

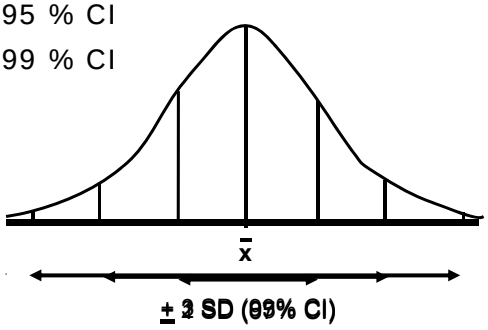
- How to Manage or Control?
- Purpose of the Method
- Method Design
  - Replicates and Independent Tests (Management of Risks and Inherent Method Variability)
- Reportable Result – Mean of Replicates, Independent Tests

Risk Management for Errors, Subjectivity & Controlling Variability

- At Least 2 Independent Determinations (Part of the Method – Qualified/Validated)
- Differences between 2 Determinations within Assay Variability
- Each Determination as Raw Data
- Mean of 2 Determinations - Reportable Result
- Controls Inherent Method Variability

Normal Distribution

- Data Must be Normally Distributed for Relying on
  - Mean
  - Standard Deviation (SD)
  - Coefficient of Variation (CV) or Relative SD (RSD)
- ± 1 SD = 67% Confidence Intervals (CI)
- ± 2 SD = 95 % CI
- ± 3 SD = 99 % CI



Understanding Inherent Variability of a Method

- SD Depends upon Numbers
- CV or % RSD Normalizes Data
- Understanding CV
  - 10% CV or RSD =
    - ± 20% Variability at 95% CI
    - ± 30% Variability at 99% CI
- If Data Not Normally Distributed, SD, CI, Not very Useful
  - Use Non-Parametric Statistics

Role of Inherent Variability

- Inherent Variability of the Method
  - Used in Setting Specifications
- Often Companies showing 5 – 10% CVs with Immunochemical Methods
  - Use Very Wide Specifications, Not Consistent with CV
  - Method Validation Data Not Reliable and Useful
  - Performed Method Validation as an Exercise without Using it for Specifications

Design of Analytical Method for "Intended Purpose"

- Method for Release to Support Specifications (80 – 120%), Method Validated with a 25% CV
  - Not Suitable for Intended Purpose
    - At 95% CI, this Method can Support 50 – 150% Specifications
- Managing Variability
  - Understanding Source of Variability
  - Random Variability – Mean of Multiple Determinations
- Method Validation Parameters (Precision & Accuracy) Based on "Intended Use", NOT on Capability of the Method

Example – Management of Variability by Assay Design Example

Virus Titration ($TCID_{50}$) in Cell Cultures (Absolute Quantitation)

- ±0.5 Log Variability Acceptable (95% CI)
  - 1 Log (10-fold) Variability – Too High
- 1 in 20 Tests Have Larger Variability
- 95% Confidence Intervals – Tests
  - 1 Test – ±0.5 log
  - 2 Tests – ±0.3 log
  - 3 Tests – ±0.2 log
  - 9 Tests – ±0.05 log
  - 20 – 30 Tests Close to True Value

Application in Design of Test for Manufacture (Formulation) & Testing

- Requirement $\geq 10^6$ $TCID_{50}$/ml
- Bulk Conc. – 9 Tests for Formulation (±0.05)
  - Example, 10^8 ($10^{7.95}$ to $10^{8.05}$)
- Formulate at 106.5 $TCID_{50}$/ml
- Passes by 1 Test – 97.5% (10^6 to 10^7)
- Release Test – 3 Tests ($10^{6.2}$ to $10^{6.8}$ 99% CI)
- Stability – 1 Test (~2.5% OOS Risk)

Method Validation

- All Validation Parameters should be Evaluated for Reportable Results
  - Reportable Results (Required Replicates, Tests as per Method Procedure)
  - Exception – Repeatability (Biological Assays)

Status of Method During Product Life Cycle

- Validated Methods
  - Release Testing of Licensed Product
    - Raw Materials, Intermediates, Final Bulk (DS), Final Container (DP)
  - Stability Program
  - Safety Evaluation – All Stages (Sterility, Adventitious Agents)
  - Viral Clearance Studies
  - Cleaning Validation

Status of Method During Product Life Cycle

- Preferred to be Validated
  - Process Validation Studies
  - Equipment Qualification
- Qualified (Scientifically Sound Providing Reliable Results)
  - Phase 1 and 2 Clinical Studies (Except Safety Assays need Validation)
  - Characterization and Other Tests (For Information) – Varying Degree of Qualification

Summary and Conclusions

- Analytical Methods play a Central & Critical Role in Drug Development and Assuring Quality
- Analytical Methods must be Selected and Designed based on Sound Science and Regulations
- Methods must be Suitable for Intended Purpose
- Same Methodology can be used in Different formats for Various Purposes – Formulation, Release & Stability


Thank You!
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