

Reference Standards for Vaccines

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Reference Standards for Therapeutic Proteins:

Their Relevance, Development, Qualification and Replacement

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Outline of Presentation

- Need for Biological Standards
 - Biological Standardization & History
- Definitions/Terminology
- Vaccine Standards – Types and Examples
- Standards for New Products
 - Selection and Calibration
- Use of Standards – Standard Curves
- Influenza Vaccine Reagents

Need for Standards for Vaccines

➤ Biological Standardization

- Biologicals Not Characterized Adequately by Chemical or Physical Means
- Biological Activity Measured by Biological Methods
- Measurement of Biological Activity Based on Probability
- Comparison of Like with Like
- Standards Based on Weight (Units), Not on Physiological Activity
- Complexities with Combination and Adjuvanted Vaccines

➤ Early History

- Paul Ehrlich Established Principles of Biological Standardization in Late 19th Century (1st Diphtheria Antitoxin Standard)
- Sir Henry Dale Expanded Biological Standards (Early 20th Century - Standard for Insulin)

Measurement of Biological Activity

- Historically as Physiological Activity
 - Frog Units, Mouse Units, Cat Units, etc.
- Titers & Absolute Quantitation
 - Potency Test of Live Vaccines, CFU, 50% End Points, PFU (Controls Included)
 - Antibody Levels, Neutralization, HAI, ELISA (Highest Dilution Providing Specific End Point)
- Calculation Against Reference Standard (Mass or Units)
 - Manages and Controls Variability
 - Inter-laboratory Comparison – Reliable
 - Better Control of Assay

Need for Reference Standards

Gupta & Siber, Biologicals 1995, 23, 71-73

Table 1. Pertussis antitoxin titres of human sera determined by Chinese hamster ovary cell assay using two different methods for calculating titres

Toxin Dose per well † (pg)	Volumes in assay (µl)			Method of determining Titre*	Pertussis Antitoxin titre of Serum No.				
	Serum	Toxin	Cells		1	2	3	4	5
120	25	25	200	Method 1	128	>1280	640	4	128
	25	25	200	Method 2	1280	>12 800	6400	40	1280
120	50	50	100	Method 1	128	>1280	640	10	128
	50	50	100	Method 2	512	>5120	2560	40	512
240	50	50	100	Method 1	128	>1280	640	8	128
	50	50	100	Method 2	512	>5120	2560	32	512
2400	50	50	100	Method 1	32	320	128	<2	40
	50	50	100	Method 2	128	1280	512	<8	160

*In method 1, the titre was calculated as the reciprocal of the highest dilution of serum added to well which completely neutralized the clustering effect of pertussis toxin. In method 2, the titre was taken as the final dilution of the serum caused by the addition of the toxin dose and cell suspension to the well.

†The minimum CHO cell clustering dose of this PT preparation was 15–30 pg.

International & US Standards for Vaccines

- International Standards
 - Historically at Statens Serum Institute in Copenhagen
 - Since 1997 at National Institute for Biological Standards & Control, UK
- US Standards (Center for Biologics Evaluation and Research)
 - 21 CFR 610.20 Standard Preparations
 - Potency
 - Antibodies
 - Antigens (Vaccines)
 - Blood Derivatives (Thrombin)
 - Opacity (Microbiology Standard to Measure Bacterial Count)
 - Individual Monographs (21 CFR 600), All Vaccines Removed from CFR

Definitions/Terminology

- International Standards (IS)
 - Defined in International Unit (IU)
 - Based on Extensive International Collaborative Study
 - Different Assays, when possible
- International Reference Preparation (IRP)
 - Same Purpose as an International Standard
 - Without a Full International Collaborative Study
 - Not Suitable as IS, Based on International Collaborative Study
- IS and IRP, Primary Standards for Calibration of National or Lab Standards and Reference Preparations

Definitions/Terminology – cont'd

- Certified Reference Material (CRM) or IS
 - Certified by a National or International Body, e.g. USP, NIST, ACS, ATCC, FDA, NIBSC, SSI, WHO
 - Used as Primary Standard
- Reference Standard or Calibrator
 - Used to generate a Standard Curve
 - Results of test samples from Standard Curve
 - CRM, IS, Reagents of Analytical Grade
- Control
 - Tested with Sample to Verify System Suitability (Validity)
 - Negative Control (Pre-Defined Criterion)
 - Positive Control – Low, Middle (Product Sample) & High
 - Acceptable Range, Predetermined (95 or 99% CI)
 - Track and Trend (Monitoring of Method Performance)

Types of Standards for Vaccines

- Potency
 - DS, DP or Formulated Monovalent Component
 - Clinical Relevance (Whole Cell Pertussis Vaccine)
 - Consistency in Manufacture (Acellular Pertussis Vaccine)
 - Antibodies (Usually Animal), Polyclonal, Monoclonal
- Clinical Serology (Helpful in Defining Protective Levels)
 - Antibodies (Usually Human)
- Other Standards
 - Opacity Standard
 - Excipients, Adjuvants, Intermediates, etc.
- Critical Reagents, Internal Controls

Common Vaccine Standards

- Diphtheria Antitoxin (Established for DAT Product, Used for Diphtheria Vaccines)
 - Developed by Ehrlich 1898, Based on Mass or Weight
 - American Standard (Units) in early 1900's
 - IS in 1922 (IU and American Units – Similar)
- Tetanus Antitoxin
 - American Standard (Units) in early 1900's
 - IS 1928; 2 IU = 1 American Unit; In 1949 Adjusted 1 IU = 1 American Unit
 - 1969 – 2nd IS; 1992 - 1st IS, TIG (Human)
- DAT and TAT Standards for Flocculation (Both US and IS)
 - In 1988 IS for Antitoxin Replaced with Toxoid
- 1st IS, Diphtheria (Schick) Test Toxin 1954
- IS for Tetanus Toxoid, Plain and then Adsorbed
- IS for Diphtheria Toxoid, Plain and then Adsorbed

Common Vaccine Standards – cont'd

- US and IS for Whole Cell Pertussis Vaccine (Correlated to MRC Clinical Trials)
- Whole Cell Cholera and Typhoid Vaccines (Did not Correlate with Clinical Efficacy)
- US Standards for Hib Antibodies (Helped Define Protective Levels)
- US Standards for Various Pertussis Antibodies
- WHO Reference Reagent Pertussis Antiserum (Human) 1st
- WHO IS Monoclonal Abs for *B. pertussis* serotyping
- US Standards for Pneumococcal Antibodies
- JN1H-3, 1st IS for Acellular Pertussis Vaccine
- JN1H-5, 1st IS for Pertussis Toxin
- Pertussis Mouse Serum (Immunogenicity Test, Consistency in Manufacture)
- Inactivated Influenza Vaccine Reagents

Standards for New Products

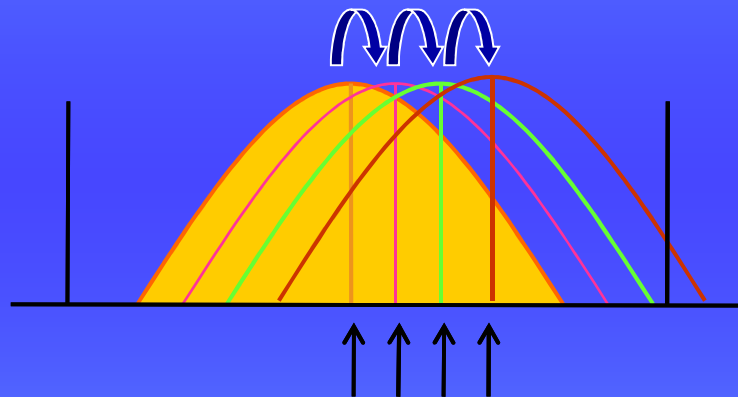
- Standards/Reference Preparations often not Available for New Products
- Regulatory Agencies Rely on Manufacturers' Standards
- Selection of Product Specific Reference Preparation
 - Evolves During Product Development
 - Discussion During Pre-IND, early IND Phases
 - Later Stage (Phase III) – Reference from Same Lot as Clinical Lot (Primary Standard)
 - Preferably Similar to Product, but may be Different

Manufacturer's Reference Standards

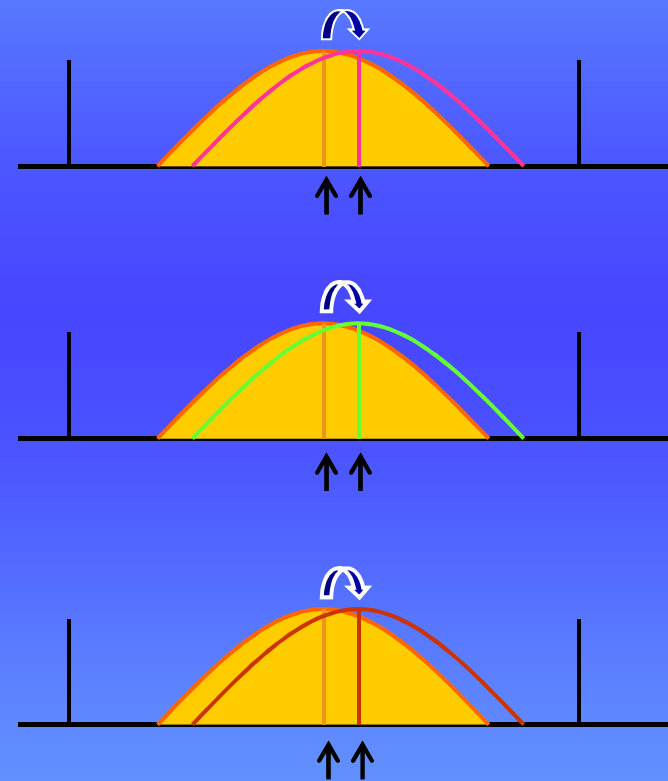
- Develop & Document Preparation, Characterization & Calibration Method (Information in Biologics License Application)
- Establish Stability, Storage, Expiration Dating
- Assure Sufficient Quantity (Shared with Regulatory Agencies)
- Be Prepared for Replacement of a Standard
 - Instability, Stock Depletion – Needs Replacement
 - Challenges in Bridging (Continuity in Activity)
 - Comparability Protocol (BLA or Supplement)
 - Calibration (Development of Primary Standard, Not Serial Calibration against Last Ref.)
- Track & Trend Parameters of Standard Curve

Calibration of Reference Standards

Calibration Against Last Lot



Calibration Against Primary Std



Factors in Selection of Antibody Standards

- Avidity of Antibodies
- Immunization Status
- Different Species
- Different Age Groups
- Use of Multiple Reference Standards
 - Species Specific
 - Preparation after Every Immunization Dose
 - Antibodies from Different Age Groups

Calibration of Antibody Reference Standards

- Calibration - No Method is Perfect
- Arbitrary Units
 - Problems in Inter-Laboratory Comparisons
- Calibration of Antibody Standards
 - For Alternate Methods in IU Against Functional Assay
 - Easy for Subjects with known Immunization Status
 - Weight ($\mu\text{g/ml}$) of Antibodies
 - Quantitative Precipitation
 - Standardized Mabs
 - Purified Immunoglobulins

ELISA Ref. Calibration in $\mu\text{g/ml}$

Gupta & Siber, J Immunol Methods 1995, 181, 75-81

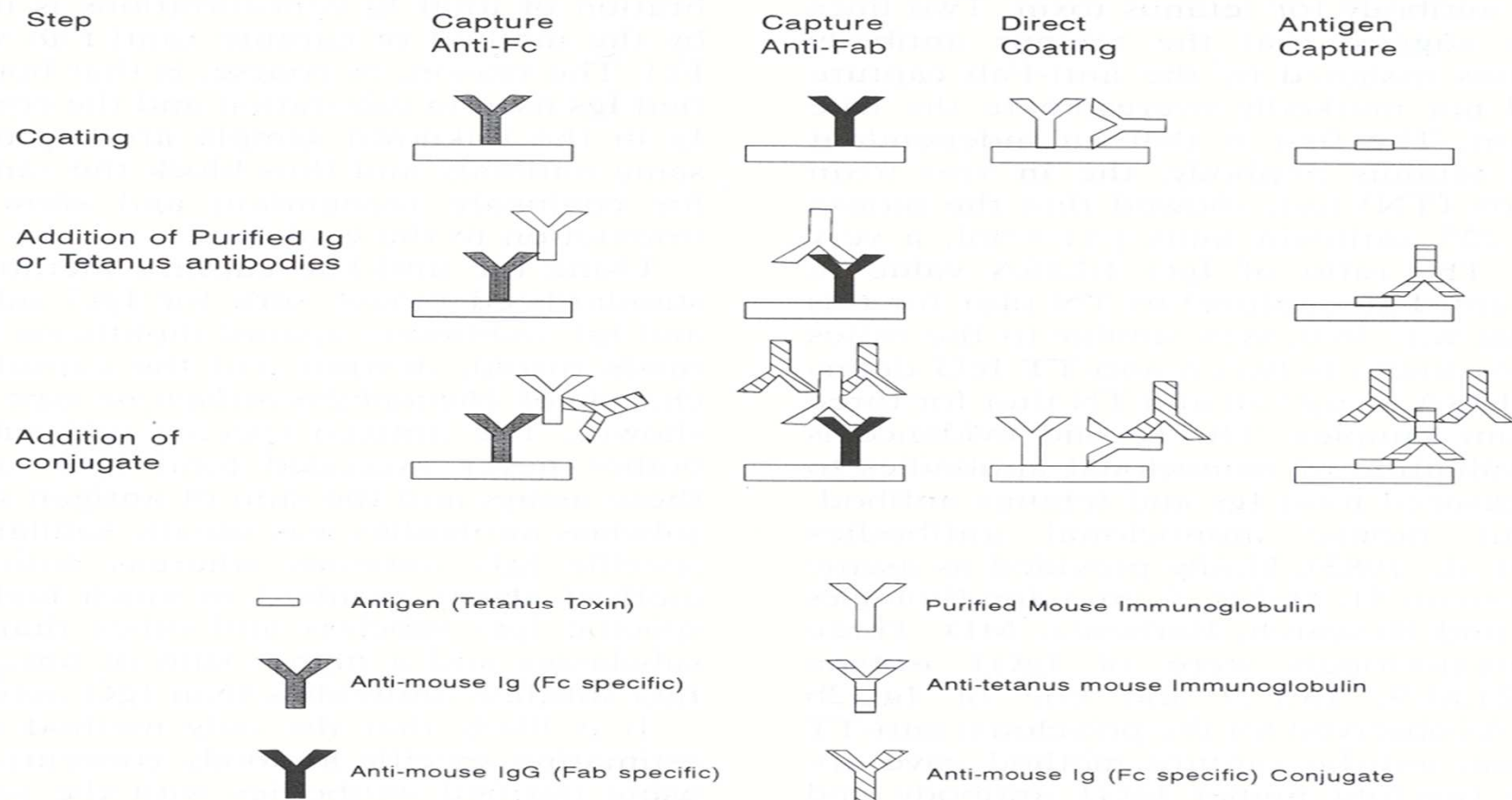


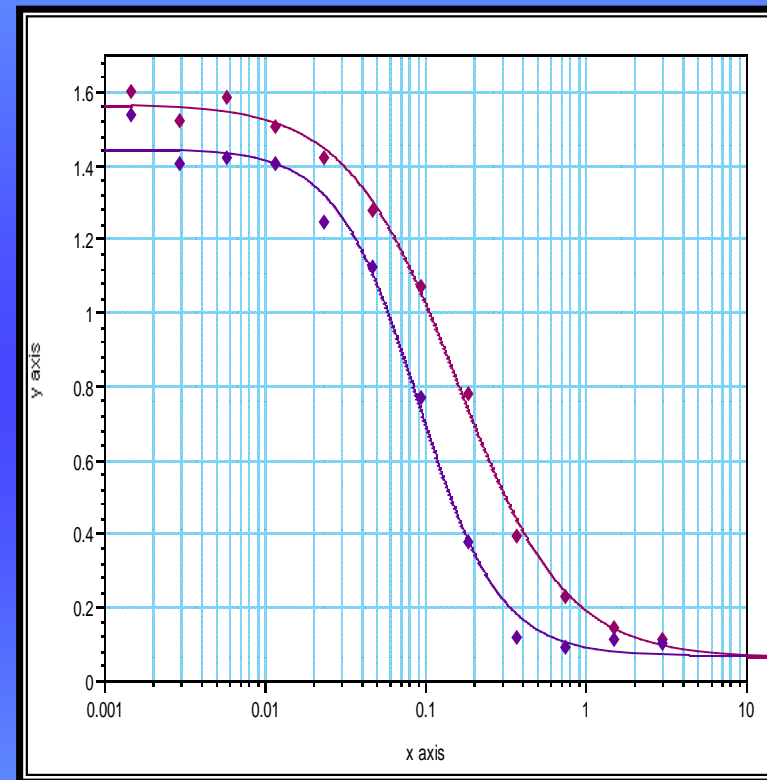
Fig. 2. Diagrammatic representation of different methods of capturing of immunoglobulins.

Standard Curve

- Linear – Log, Probit (Linearity is Important)
- Immunoassays - Nonlinear, Sigmoid Curve
- Non-linear Statistical Models
- Calculation of Results from Standard Curve
 - Avoid Extrapolation
 - Parallelism is Important
 - Use Linear Part of Curve above Background

Four Parametric Logistic Equation

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



Evaluation of Linearity & Parallelism – ELISA & Other Immunochemical Methods –

- Immunochemical Methods Often Run Full Dose Response (D/R) Curve for Sample
 - Results Determined from Multiple Dilutions
 - Linearity & Accuracy of Results Depend Upon Parallelism of Std and Test D/R Curves
 - Parallelism Depends Upon Appropriateness of Reference Standard
 - Affinity of Antibodies
 - Immunization Status
 - Age Group

Evaluation of Linearity & Parallelism – ELISA & Other Immunochemical Methods –

- Full Dose-Response Curves for Standard & Sample
- Results from 3 or more dilutions (Rarely 2)
- Mean of Results from Multiple Dilutions
 - Variability of Individual Results from Mean Not More than Inherent Variability of Method (<20%)
 - Evaluate CV (Set Specs)
 - If Higher Variability, Exclude 1 Result (At Least 2 Results)
- If non-Parallelism, Set Rules
 - For example, Value at 30% of Maximum Response

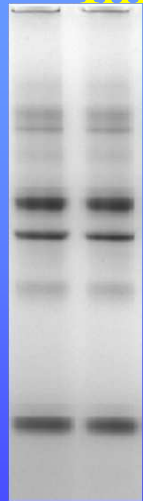
Generation of Influenza Vaccine Potency (SRID) Reagents

- Every Year CBER Generates & Provides Potency (SRID) Reagents to Manufacturers
- Strain Specific Hemagglutinin (HA) Antibodies
 - Generated in Sheep to Purified HA
 - HA from Manufacturers and/or Generated in-house
- CBER Reference Antigen
 - Primary Liquid Standard – Total Protein & SDS-PAGE
 - Lyophilized Standard – Calibrated Against PLS by SRID
 - Calibration by CBER, NIBSC, TGA, NIID (WHO – ERLs)

Application of New Technologies to Generation of Potency Reagents

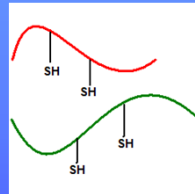
- Ion Chromatography for Kjeldahl Method
 - Accurate Determination of Total Protein on PLS using Small Amount of Sample
- MALDI TOF/TOF for Identity and Purity
 - Purified HA for Inoculation of Sheep
 - PLS for Accurate Determination of HA Content
- RP-HPLC and SE-HPLC
 - To Monitor Reference Antigen Characterization during Lyophilization

Influenza Antigen Protein Identification by MALDI TOF/TOF

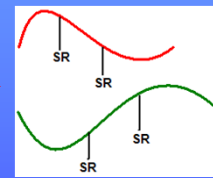


Individual Protein Bands Excised and Washed.

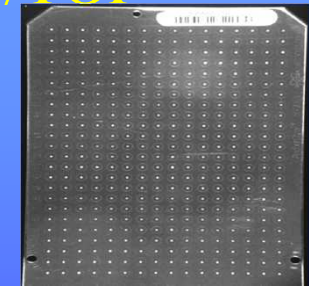
Reduction



Alkylation



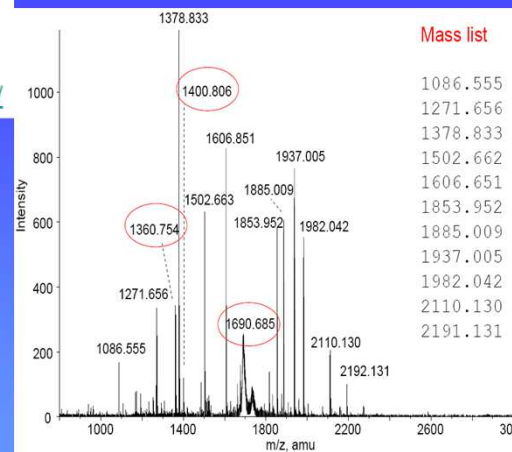
Trypsin Digestion



Tryptic Peptides Spotted on to MALDI Plate with Matrix (Alpha cyano-4-hydroxy-cinnamic acid)

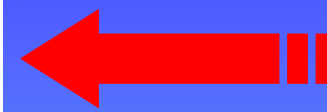


ABI 4800 MALDI 24 TOF/TOF

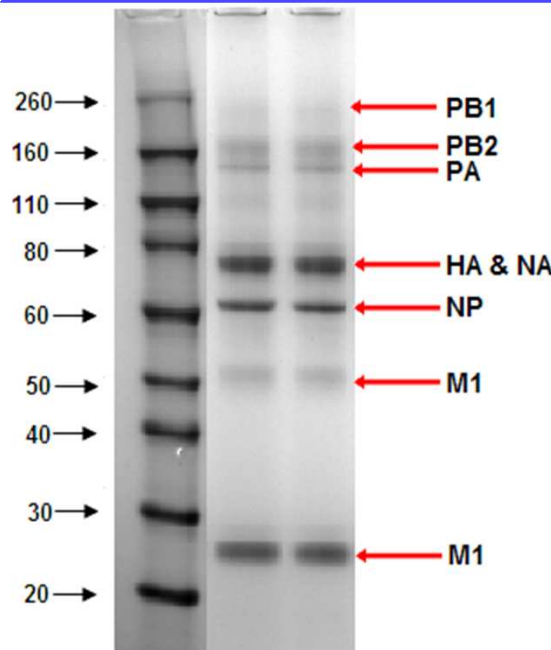


MS/MS spectrum

MATRIX SCIENCE
Matrixscience: Mascot
<http://www.matrixscience.com/>



Database search



Summary

- Biological Standards are Essential
 - Promote Consistent Product Quality among Manufacturers
 - Help in Defining Protective Levels of Antibodies
 - Facilitate Biological Method Validation Studies
- Establishment of a Primary Standard is Important
 - Provides Benchmark for Replacement & Calibration of New Standards
 - Maintains Continuity in Activity (Units)
- Coordination in Developing Standards for New Products is Beneficial

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