

Immunogenicity: Selection and use of standards for immunogenicity assays

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Session 1: Understanding the attributes required for reference standards
(primary, working etc.) and the purpose of their use

Immunogenicity

- Immunogenicity: property of a substance to *induce* an immune response. It can be inherent to a drug substance, relative to the “host”, stimulated/enhanced by accompanying substances in a drug product (formulants and impurities), and dependent upon many other factors
- Anti-Drug Antibodies (ADA): host antibodies that bind to epitopes on the drug molecule.
 - Binding antibodies (BAb): ADA detected by their ability to bind to the drug
 - Neutralizing antibodies (NAb): ADA detected by their ability to inhibit drug activity
- Mechanisms:
 - Normal immune response against ‘non-self’/neoantigens
 - Reaction to non-human drug products in humans
 - Reaction to human protein drug products in animals
 - Breakdown of immune tolerance
 - Reaction to human protein products in humans

ADA detection platforms

- Radioimmunoprecipitation (RIA, RIPA)
- Immunoassay (e.g., ELISA)
 - Direct binding
 - Bridging
- Electrochemiluminescence (e.g., MSD)
 - Direct binding
 - Bridging
- Label-free detection platforms
 - Surface plasmon resonance (SPR): e.g., Biacore™
 - Biolayer interferometry (BLI): e.g., Octet
- Others ... (Gyrolab, AlphaLISA, etc)

Components of ADA detection methods

- Critical Reagents:
 - Antibody - ADA Positive control
 - Antigen (Drug) – naked molecule, conjugated molecule (based on assay platform)
 - Drug target – needed for NAb assays
- Other Reagents:
 - Blocking reagent
 - Assay diluent

What is a Standard”?

- = A “reference” standard; a universal comparator.
- FDA’s Bioanalytical Method Validation (May 2001) Guidance: “an authenticated analytical reference standard of known identity and purity should be used to prepare solutions of known concentrations. *If possible, the reference standard should be identical to the analyte*”.
- USP: “[material] selected for high purity, critical characteristics, and suitability for the intended purpose”.
- WHO: “...defined biological activity expressed in an internationally agreed unit” **AND** “well-characterized preparations as global measurement standards against which batches of biological products are assessed...”.

Opportunity for standard preparations of critical reagents in ADA Methods

- For establishing comparable ADA test methods between innovator and biosimilars so that immunogenicity rates can be compared
- To enable an understanding of methods used by sponsors of same-class drugs? (sensitivity, drug interference level, drug target interference level, etc)
 - FDA's general caution in labels that immunogenicity rates of drugs should not be compared because the observed incidence of antibody positivity in an assay may be influenced by several factors, including sample handling, timing of sample collection, concomitant medications and underlying disease.
 - **Despite this caution, we know that many compare rates of ADA incidence between same-class drugs....**
 - So, can something be done to enable a comparison of method sensitivity?

Reference standards for ADA methods?

- Critical Reagents:
 - Antigen (Drug) – YES
 - Drug target – YES
 - ADA Positive control – NO, how do we choose the right type of control? (or YES, *with caveats*, and may require more than one type of positive control)
 - Drug naïve ADA negative samples from healthy individuals and those with various disease states (for cut point determination)
- ADA positive control(s) for each assay could qualify as a “reference standard”. But it is not going to be identical to the clinical “spectrum of analyte”.
- Positive control is seemingly THE hurdle for forming the basis for a uniform reporting system

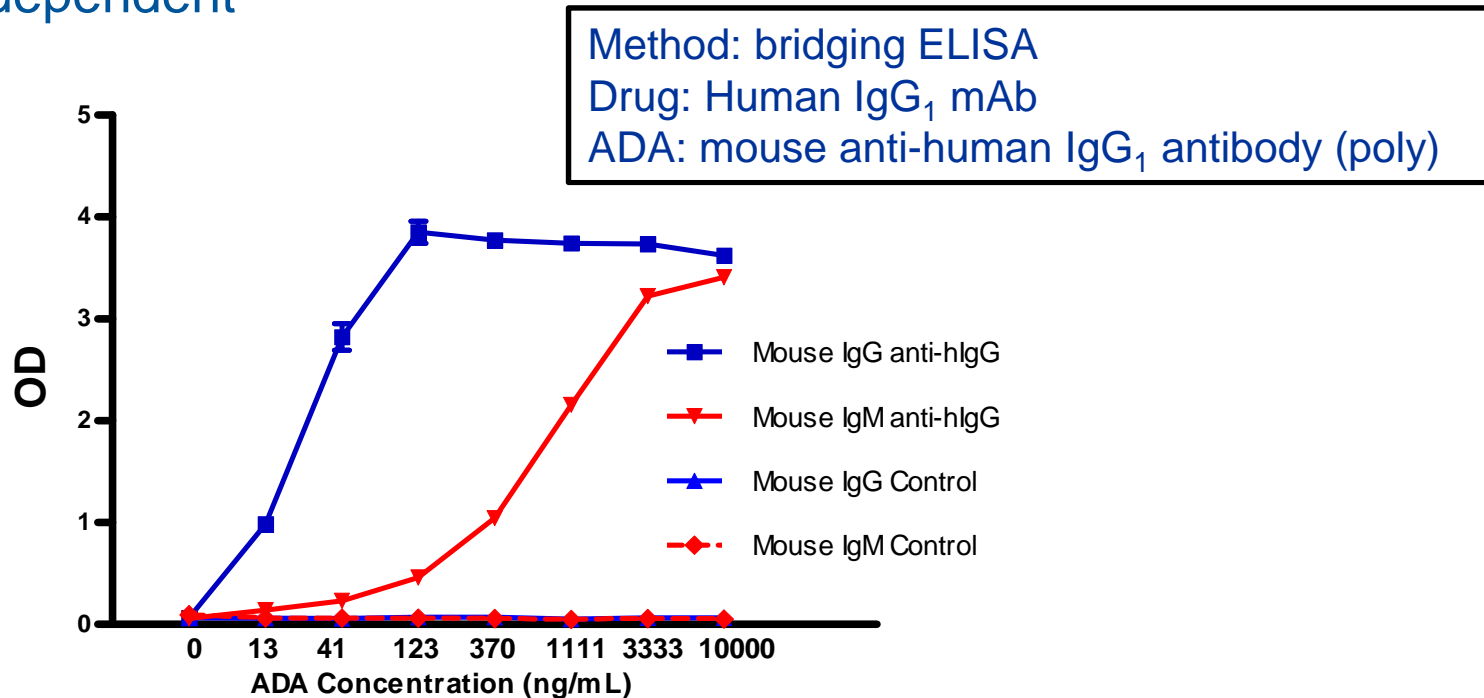
ADA detection methods – a common issue

- Assay development depends upon availability of analyte (a positive control)
- *ADA is not “an analyte”; it is a spectrum of analyte/reactivity*
 - Species specific
 - Epitope specific
 - polyclonal (probably), varying avidities
 - Heterogeneous
 - In humans, ADA could be expressed as IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgE; Other species produce other isotypes
- Assay performance is optimized for THE positive control analyte on hand; we are lucky when more than one positive control is available.
- Not quantitative; at best, can be described as *quasi-quantitative*.
- The positive control is often a polyclonal antiserum or a monoclonal antibody.
- So how good is a test method based on a single analyte, in detecting “a spectrum of analyte” in the subjects?
- Can we select one analyte as the Standard?

ISSUES: Example 1

Detection of IgM and IgG can differ in the same method

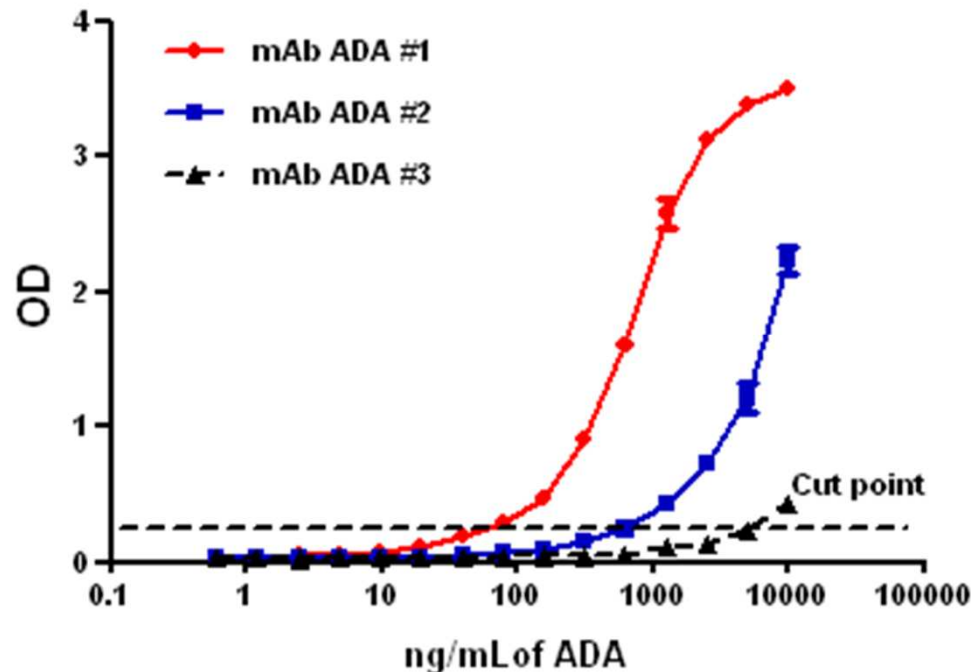
- A bridging ELISA is theoretically species-independent and isotype-independent



- Drug-specific IgM controls are often not available (what about IgA and/or IgE controls that may be needed in some situations)
- Even if they were available, does each control represent the “a spectrum of analyte”?

ISSUES: Example 2

Different ADA often produce non-parallel dilution curves



Method: ELISA

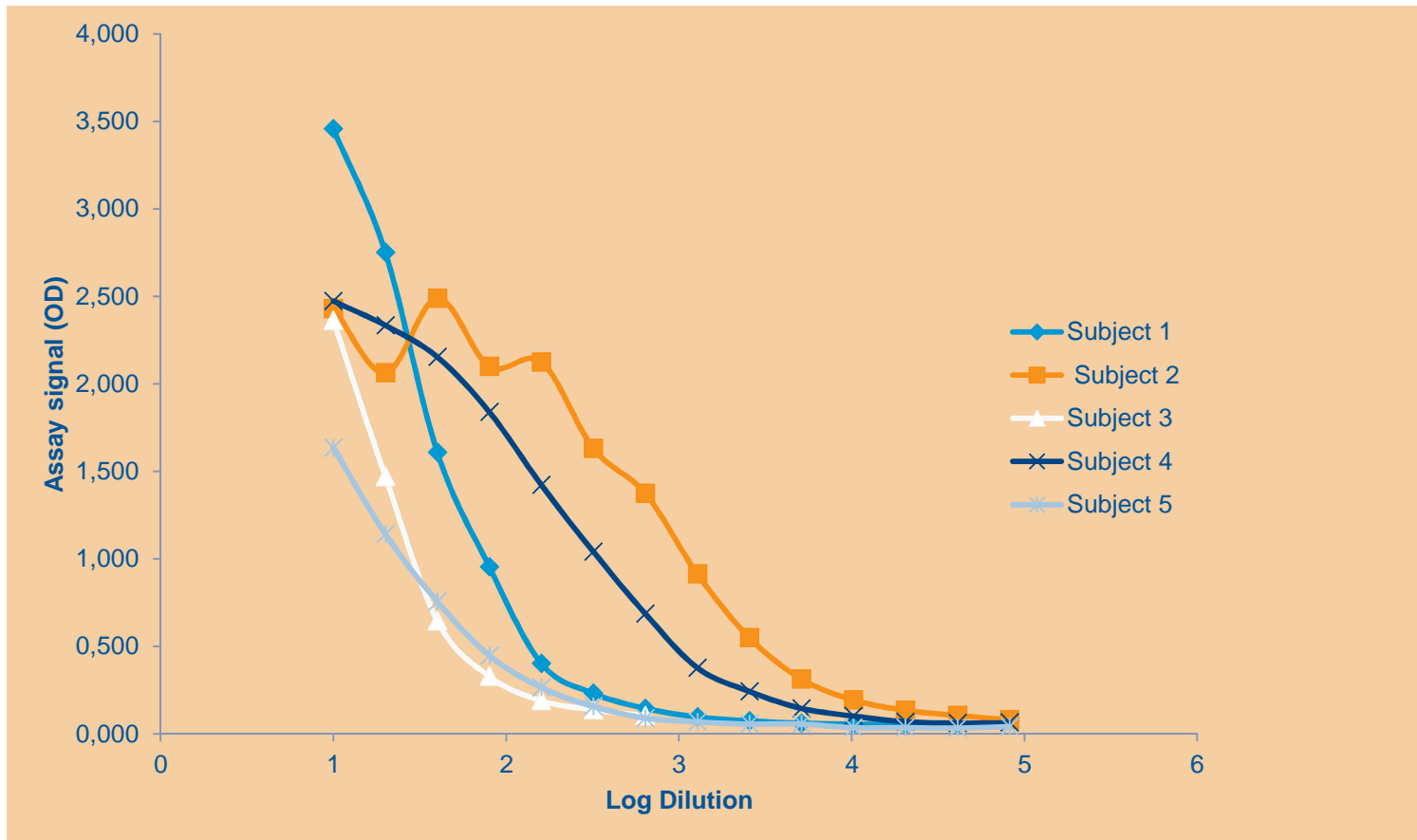
Drug: Human IgG₁ mAb

ADA: human anti-idiotypic monoclonal ADA

- Which ADA should be chosen as the optimal positive control for a qualitative/quasi-quantitative method of ADA detection?

ISSUES: Example 3

Clinical ADA samples also show variable dilution curves



- When the analyte in clinical situations is varied, how to identify a positive control reference standard?

ISSUES: Example 4

Detection of ADA with disparate affinities differs in the same method

Anti-Id ADA	KD (nM)	EC50 in Bioassay
# 1	1.6	2.87ng/mL
# 2	3	854.2ng/mL
# 3	3	608.3ng/mL
# 4	3.5	69.03ng/mL
# 5	3.6	542ng/mL
# 6	3.8	359.4ng/mL
# 7	4.4	236.0ng/mL
# 8	4.7	144.7ng/mL
# 9	4.9	2344ng/mL
# 10	5.5	818.70ng/mL
# 11	6.6	36.86ng/mL
# 12	8.3	1µg/mL
# 13	8.5	3261ng/mL
# 14	10.3	391.5ng/mL
# 15	10.8	201.4ng/mL
# 16	11.8	1793ng/mL
# 17	12.7	1324ng/mL
# 18	14.3	No Inhibition

Method: cell-based bioassay

Drug: Human IgG₁ mAb

ADA: human anti-idiotypic monoclonal ADA

- Which ADA should be chosen as the optimal positive control?

ISSUES: Example 5

Sensitivity of detecting ADA with disparate binding affinities differs across methods

Anti-Id ADA	K _D (nM)	Sensitivity (ng/mL) in neat serum		
		MSD	ECLIA	Octet-QK
Cyno Poly	NA	6	1	130
Mab #1	6.0	24	12	500
Mab #2	6.2	781	391	1000
Mab #3	6.5	195	391	1000
Mab #4	6.6	781	781	1000
Mab #5	11.6	391	391	1000
Mab #6	12.2	6250	1563	5000
Mab #7	14.0	3125	1563	1000
Mab #8	50.0	6250	3125	2000
Mab #9	65.8	12500	6250	2000

Method: 3 LBA platforms

Drug: Human IgG₁ mAb

ADA: human anti-idiotypic monoclonal ADA AND an affinity purified polyclonal ADA from cynomolgus monkey antiserum






- Which ADA should be chosen as the optimal positive control?

Availability/Choice of positive control is critical

- The positive control greatly influences:
 - Assay development
 - The choice of assay platform
 - Method sensitivity
 - Drug tolerance
 - How well a qualified/validated method performs in ADA detection
 - Detection of low levels (sensitivity) of various low affinity and high avidity antibody responses
 - Drug tolerance of various low affinity and high avidity antibody responses
 - *Are we developing the right methods for detecting clinically relevant ADA?*

What is a “Standard”?



- 1. something considered by an authority or by general consent as a **basis of comparison**  Meets reference standard expectation
- 2. an object that is regarded as the **usual or most common size or form** of its kind.  Meets reference standard expectation
- 3. an **average or normal requirement**, quality, quantity, level, grade, etc.  Meets reference standard expectation
- 4. **a rule or principle** that is used as a basis for judgment.  Could standardizing data interpretation approaches benefit us?
- 5. ...those morals, ethics, **habits**, etc., established by authority, custom, or an individual as acceptable.  Could standardizing methodological approaches benefit us?



Methods for the same analyte also differ in decision-enabling criteria

- Eg. IFN- β specific BAB detection by ELISA methods*

Type ^a and concentration of IFN	Validation/cut-off	Reference
IFN- β -1a or 1b/0.2 μ g	Mean + 3 \times SD of normal	Perini <i>et al.</i> (2004)
IFN- β -1b/concentration not given	NAB (MxA induction)	Kremenchutsky (2003)
IFN- β -1a and 1b/1.5 μ g/ml	NAB assay	Pachner <i>et al.</i> (2004)
IFN- β -1a/1b/human IFN- β	Mean + 3 \times SD of normal	Bellomi <i>et al.</i> (2003)
IFN- β -1b/1000 IU/ml	NAB assay	Mayr <i>et al.</i> (2003)
IFN- β /1 μ g/ml	2 \times background of uncoated wells	Slavikova <i>et al.</i> (2003)
IFN- β -1a/1 μ g/ml	Mean + 3 \times SD of baseline sera	Monzani <i>et al.</i> (2002)
IFN- β /1.2 μ g/ml		
IFN- β -1a/1 μ g/ml	3 \times OD of background	Vallittu <i>et al.</i> (2002)
IFN- β -1a and 1b/1 μ g/ml	Arbitrary (OD > 0.5)	Fernandez <i>et al.</i> (2001)
IFN- β -1a and 1b/10 ⁴ U/ml	Standard curve ^b	Kivisakk <i>et al.</i> (2000)
IFN- β -1a and 1b/1–312 ng per well	Mean + 2 \times SD of controls	Antonelli <i>et al.</i> (1999)
IFN- β -1b/5000 IU per well (ELISA)	NAB assay/detection limit of WB	Deisenhammer <i>et al.</i> (1999)
IFN- β -1b/2.5 μ g per gel		
IFN- β -1b/2 μ g/ml	Control placebo samples/39 binding units	Pungor <i>et al.</i> (1998)
IFN- β -1a and 1b/1000 IU/ml	Mean of control + 2 \times SD	Khan and Dhib-Jalbut (1998)
IFN- β -1b/1 μ g/ml	Mean of control + 3 \times SD	Ferrarini <i>et al.</i> (1998)

*Source: Sorensen et al (2005), European Journal of Neurology, 12: 817-827

Conclusions

- Standardization of a positive control is challenging. Not impossible, if we can achieve consensus on accepting the caveats.
- To enable the formation of a basis for a *uniform reporting system for immunogenicity data*, the “selection and use of standards for immunogenicity assays” should not only include standardization of critical reagents, but also:
 - Standardized assay procedures
 - Standardized assay decision criteria (cut points)
 - Standardized approach to sensitivity determination
 - Standardized data analysis and presentation

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