Critical Reagents: Update on the Guidelines and Current Practice in Industry for ADA Assays

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on behalf of the EBF

EBF Training day

Critical Reagents for LBA

Altis Grand Hotel, Lisbon - 14 May 2018

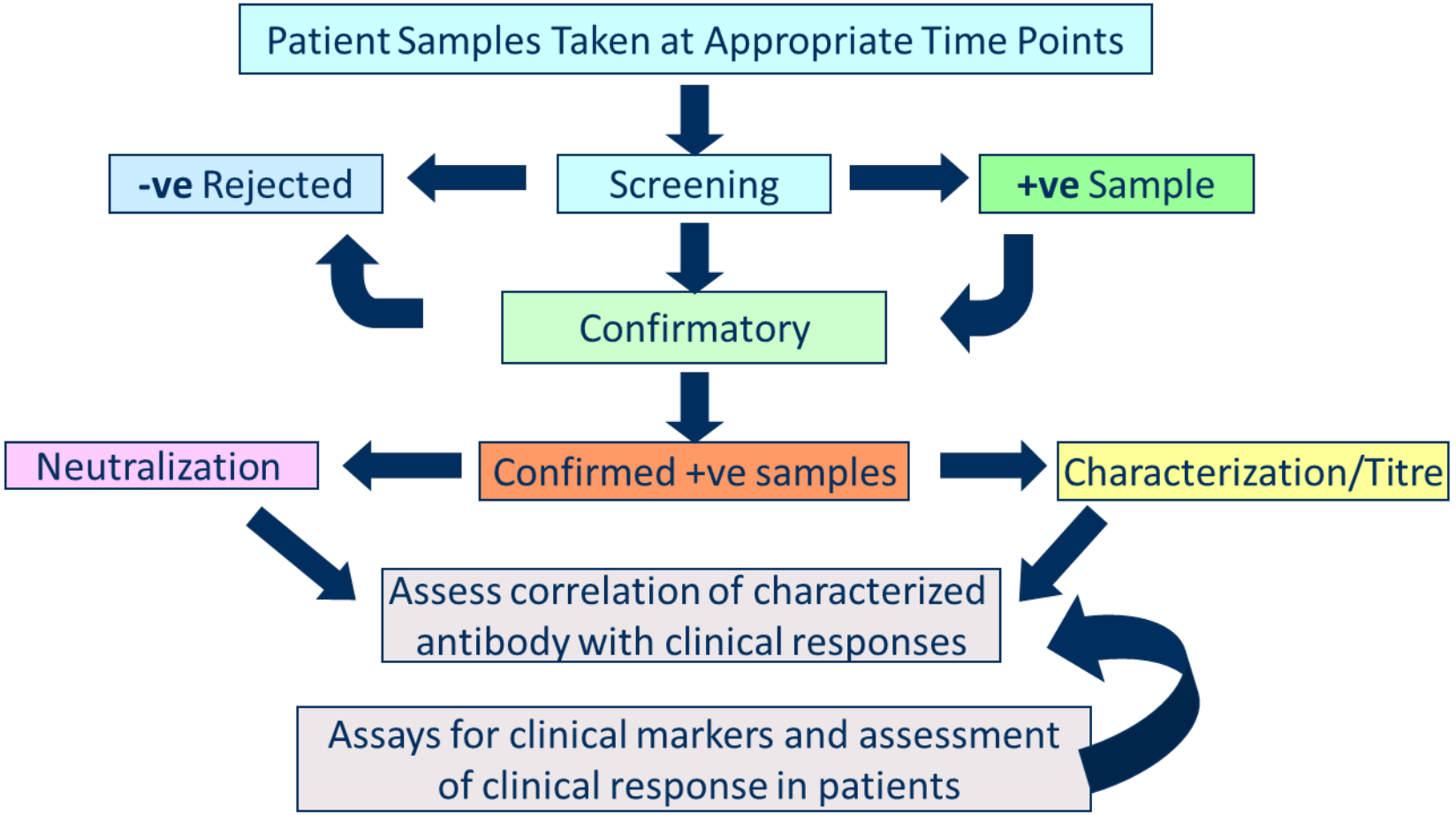
When thinking about critical reagents we need to consider the assay

- Assays for detecting anti-drug antibodies have some key differences to PK assays

Description	PK Assay	ADA Assay
Measurement Type	Quantitative	Qualitative Tiered approach – multiple assay types
Calibrator	Well characterised reference standard Used in sample testing	No standard curve in sample testing Use of a "surrogate" control, usually generated in animals Negative control
Sensitivity	Defined based on mass unit concentration	Defined in relation to the cut point of the assay
Reagents	Usually a sandwich immunoassay In-house generated or commercially sourced	Depends on the assay format and tier of testing (LBA/CBA/SPR etc.)

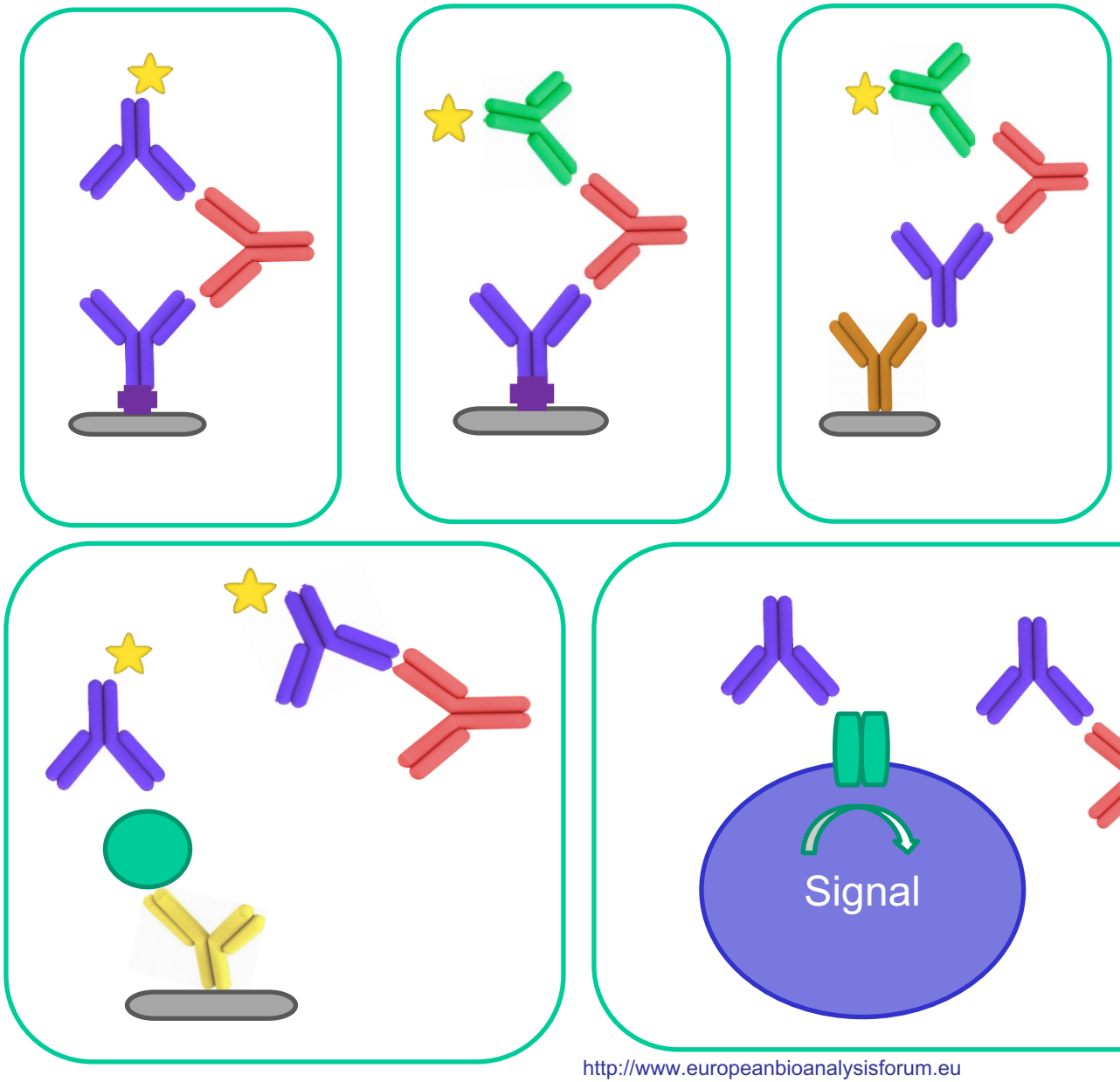
- Due to the qualitative nature of ADA assays, critical reagents changes could be more impactful

ADA assays follow a step-wise approach and each tier may have a different consideration for critical reagents



Not all tiers may be adopted for preclinical, depending on the molecule risk assessment

ADA assays come in many flavours



ADA

Drug

Labels

Anti-isotype Ab

Target

Anti-target Ab

Cell with receptor

Anti-drug Ab (not ADA)

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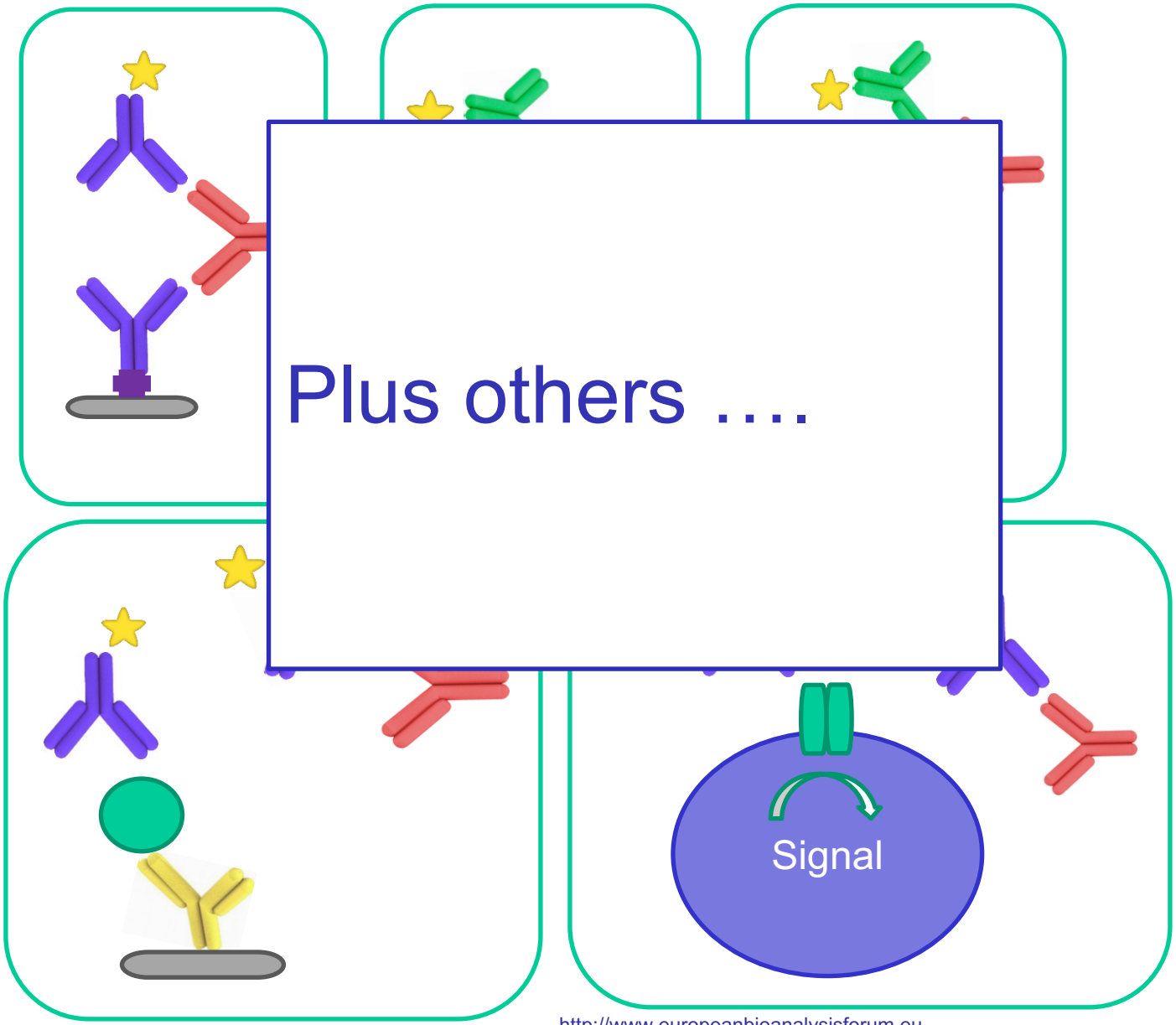
ADA assays come in many flavours



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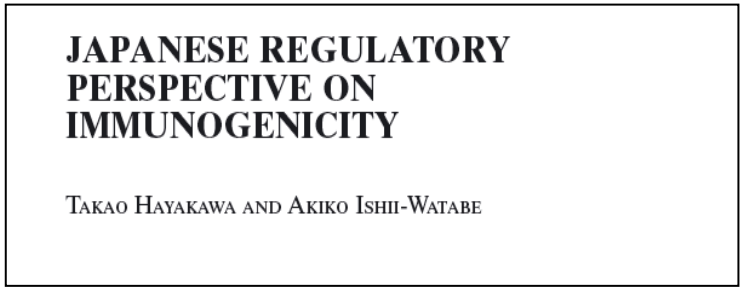
Immunogenicity guidelines/guidances

- **EMA and FDA are the only Health Authorities that have issued specific guidance**
- **EMA**
  - Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use (**2012** - EMA/CHMP/BMWP/86289/2010)
  - Guideline on similar biological medicinal products (**2015** - CHMP/437/04 Rev 1)
  - "Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins" (**2008** - EMEA/CHMP/BMWP/14327/2006)
  - Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins (**2017** - EMEA/CHMP/BMWP/14327/2006 Rev 1)
- **FDA**
  - "Immunogenicity Assessment for Therapeutic Protein Products" (**2014**)
  - "Scientific Considerations in Demonstrating Biosimilarity to a Reference product" (**2015**)
  - "Assay Development for Immunogenicity Testing" (**2009 – draft**)
  - "Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products" (**2016 - draft**)

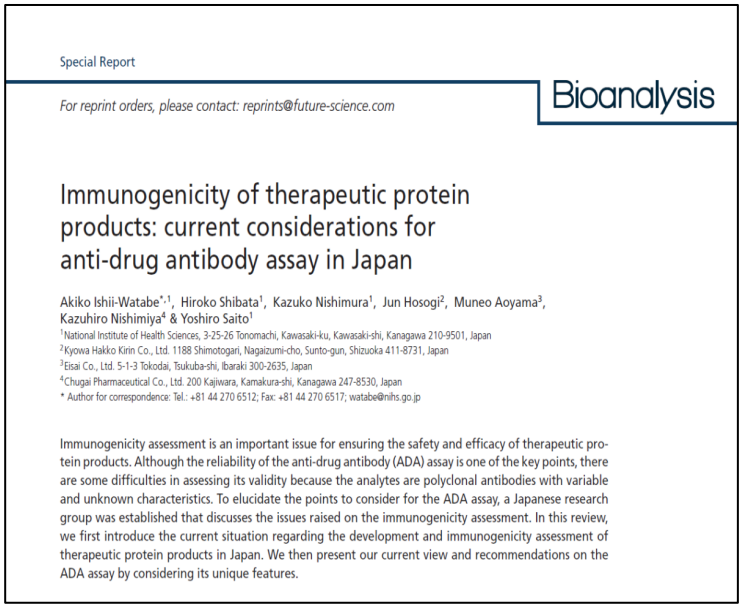
Other regions

- Japan
  - Two publications
- Brazil
  - Applies FDA/EMA
- China
  - CFDA plans to form consensus among industry and research by publishing a series of “white papers”
  - After industry consensus is formed, guidance will be planned



JAPANESE REGULATORY PERSPECTIVE ON IMMUNOGENICITY

TAKAO HAYAKAWA AND AKIKO ISHII-WATABE



Special Report

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Bioanalysis

Immunogenicity of therapeutic protein products: current considerations for anti-drug antibody assay in Japan

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Immunogenicity assessment is an important issue for ensuring the safety and efficacy of therapeutic protein products. Although the reliability of the anti-drug antibody (ADA) assay is one of the key points, there are some difficulties in assessing its validity because the analytes are polyclonal antibodies with variable and unknown characteristics. To elucidate the points to consider for the ADA assay, a Japanese research group was established that discusses the issues raised on the immunogenicity assessment. In this review, we first introduce the current situation regarding the development and immunogenicity assessment of therapeutic protein products in Japan. We then present our current view and recommendations on the ADA assay by considering its unique features.

So what does guidance say about critical reagents for ADA assays?

Perspective of EMA

- EMA (2008) Annex 1
  - *"An antibody standard/reference material/control" or "pooled human serum" "that is stored appropriately"*
  - *"Reagents need to be qualified and acceptance specifications set, at least for those,* ***which are most important****."*
  - *"… the use of relevant biological standards and/or well characterized positive and negative controls.* ***These reagents function as critical reagents*** *and are essential for assay calibration and validation."*

- EMA (2012)
  - *"Generation of* ***positive control sera is in general a critical issue*** *for immunogenicity studies for mAbs."*

Perspective of FDA

- FDA (2009)
  - *"FDA believes **positive control or QC samples are critical**"*
  - For detection reagents - *"**the nature of the detection is critical**"*

- FDA (2014)
  - *"Sponsors should develop and implement sensitive immunoassays commensurate with the **overall product development program**."*

- FDA (2016)
  - *"FDA recommends that sponsors **generate and reserve** positive control antibody solution for use as a quality or system suitability control."*
  - *"FDA recommends **storing patient samples** in a manner that preserves antibody reactivity at the time of testing.*
  - *"For example, changes in temperature, incubation times, or buffer characteristics, such as pH and salt concentration, can all impact assay results. The **complexity of bioassays** makes them particularly susceptible to variations in assay conditions, and it is essential to **evaluate and optimize parameters such as cell passage number, incubation times, and culture media components**."*

What is the industry perspective?

- Shankar *et al.* (2008)
- *"…. should assess which conditions are likely to vary and design appropriate tests to examine the parameters that are deemed critical. These may include* ***changes in microtiter plate lots, reagent lots****….."* (Robustness)
- *"Stability characterization may also include stability of* ***assay-critical reagents such as the quality controls, the coated assay plate or chip (as applicable), and other critical reagents (such as conjugates).*** *However, this is a business decision rather than a stipulated validation characteristic because the* ***ADA assays are stability indicating (i.e., loss of stability of critical reagents can be detected by poor assay performance, assay failure, monitored via the system suitability control).****"*
- *"When there are changes in critical method components …. An* ***assay revalidation may be required*** *…. The revalidation may* ***cover some or all validation characteristics*** *…. Use of lots or* ***batches of assay critical reagents that are different from those used in pre-study validation do not require assay revalidation, but must be supported by appropriate experimental qualification to ensure maintenance of system suitability****."*

Further industry perspectives

- Mire-Sluis *et al.* (2004)
- *"If there is only a single supplier of a critical reagent then it is advisable to consider some contractual relationship … test multiple lots … It is recommended that the* ***quality acceptance criteria and standard operation procedures for testing be applied to critical reagents included in the assay****, to ensure quality and continuity when replacements are required."*
- *"The stability of the* ***coat and conjugate antibodies should be investigated*** *…. Acceptance criteria for the stability of the reagents are dependent on the influence of that reagent on the performance of the assay and should be justified."*

- Staack *et al.* (2013)
- Characterisation of in-house and commercial reagents is recommended and changes can impact the assay
- Potential for aggregation and stability challenges with labelled reagents

Further industry perspectives

- King *et al.* (2014) - GBC L4
- Focused on PK, PD and ADA assay reagents, stability and management
- *"**Lack of commonality** in practices in how to manage changing critical reagents, including **major and minor lot changes**, documentation and **criteria** for performance testing of reagent stability and lot-to-lot continuity."*
- *"may be critical to the performance of ADA a**Even assay buffers or blocking reagents** ssays."*

- O'Hara *et al.* (2013)
- Consideration of masking epitopes for labelled reagents
- Biosimilar molecules and the use of one ADA assay or two
- When using two distinct assays (separate PCs – one for innovator and one for biosimilar), *"requires **thoughtful and thorough characterization** of all assay reagents to ensure any bias detected in immunogenicity detection between the two molecules is not assay-related."*

What is important in terms of critical reagents for immunogenicity assays?

- Items that affect PK assays
  - Identity, storage, expiry/re-test dates, documentation, trending analysis
- Some specific considerations for ADA assays
  - Positive/Negative controls
  - Drug or specific drug domains (capture/detection, confirmatory, stimulus)
  - Conjugated reagents
  - Coated plates, CDs, beads, chips
  - Blockers/buffers (if they impact)
  - Matrix for dilutions in titre assays
  - Cells for CBAs
- Characterisation, especially labelled reagents, may be beneficial
- Changing of lots, depending on the reagent, may need special consideration for immunogenicity assays
  - How to bridge?
  - How to titrate?
  - How to deal with cut points?
- Life Cycle Management (LCM) for ADA assays that are used post-marketing

Summary

- Immunogenicity assays are not PK assays
- Many formats available, often depending on the tier of analysis
- Re-testing of ADA may be required at regulatory request at BLA submission
- Only EMA and FDA have produced guidance documents covering immunogenicity
- There is little direction given to the subject of critical reagents specifically for immunogenicity assays within said guidance
- Industry has discussed through publications and presentations
- Considerations:
  - Reagents that are critical depending on the ADA assay format, stage of development and tier of analysis
  - Characterisation of reagents and considering potential aggregation or stability issues
  - Data trending
  - Documentation
  - Biosimilars using two distinct assays
- LCM will be important for assays post-marketing
- Going forward, guidance on what constitutes a critical reagent for immunogenicity assays, and how to deal with changes (major and minor) would be welcomed

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Questions