

**Revised Immunogenicity Guideline:  
Assays and methods-  
Presentation of the draft guideline and  
introduction of the topics for discussion**

**Robin Thorpe & Meenu Wadhwa**

# Revised Guideline: Differences from original

- Condensed; much 'general' and background information removed/shortened.
- Text on assays from Annex 1 deleted.
- Brought in line with the mAb immunogenicity guideline.
- Includes risk-based approach.
- Takes account of experience gained with assessing immunogenicity of biotherapeutics over the past 10 years, since the original guideline was drafted.
- Integrated summary of immunogenicity

# Immunogenicity testing

- Risk-based Approach - For each product, consider the 'risk' and conduct studies to address the risk and the severity of its potential
- Develop an integrated analysis strategy and study plan (incl sampling) relevant for the intended treatment. This is critical for elucidating the clinical relevance of immunogenicity data.
  - Carefully designed studies as part of clinical trials
  - Assays for antibodies - likely to evolve & be refined during development BUT assays for pivotal clinical trials and for post-marketing studies are expected to be validated.
  - Sampling points (incl baseline), frequency of sampling, sample volumes, processing/storage
  - Methods for assessing clinical response

Every product needs to be evaluated for immunogenicity individually and appropriate strategy adopted for each development programme

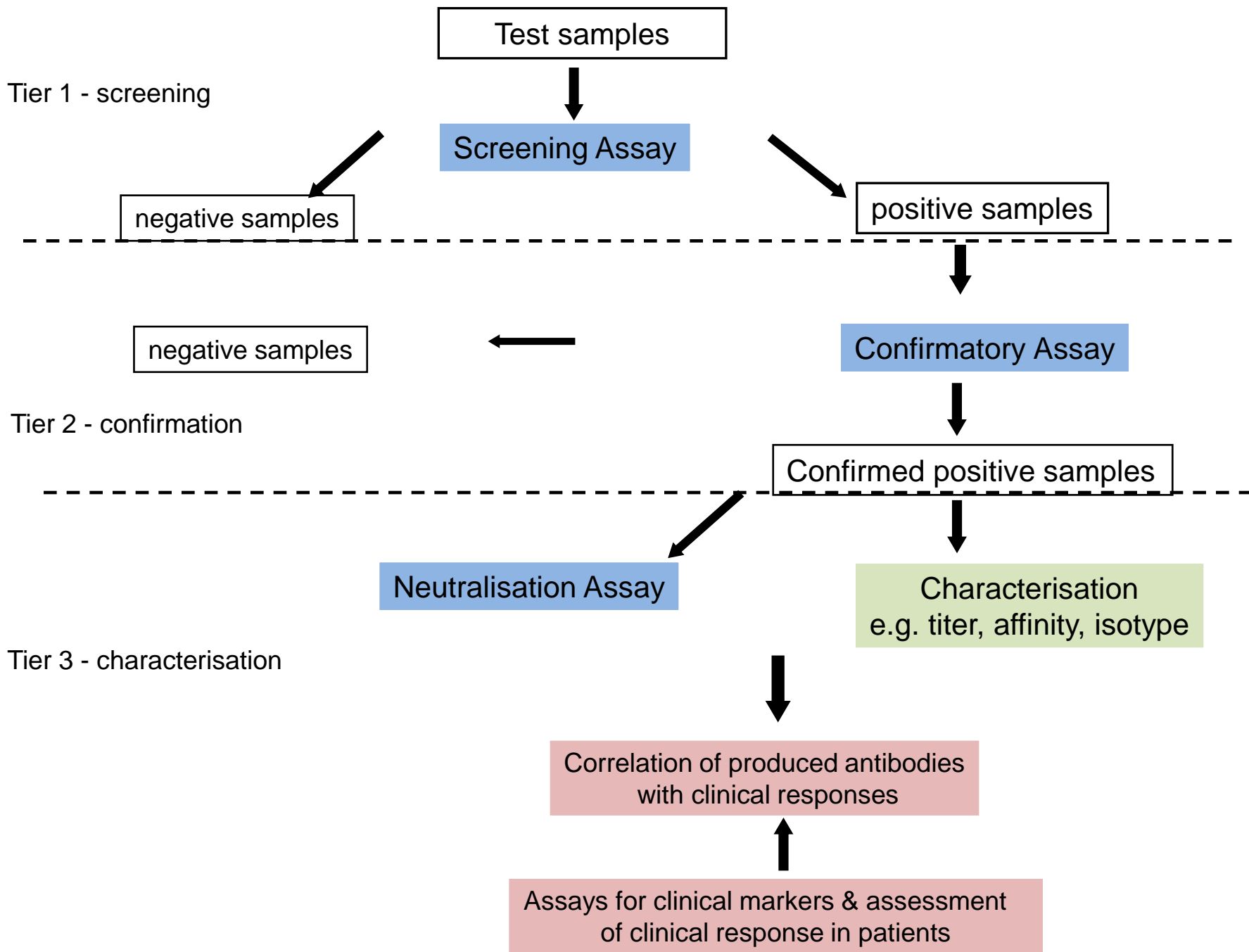
# Immunogenicity testing

- Although assay design, strategy & extent of testing are likely to vary between products, certain key elements need to be addressed in designing immunogenicity assays for application during clinical testing -
- **Sensitivity** - Sufficiently sensitive assays to detect clinically relevant levels of antibodies
- **Interference** – Assay results should not be confounded by matrix/target interference or from residual product. Any interference needs to be evaluated and strategies to minimise/overcome this implemented
- **Biological/Functional consequences** – Since induced antibodies can have multiple biological effects e.g., neutralizing activity etc, assays should be designed to detect these consequences.

# Strategy and Antibody Assays

A multi-tiered approach

- Screening assay for identification of antibody positive samples/patients.
- Confirmatory assay for minimising false positive results following the initial screen. Usually by addition of excess therapeutic and comparing spiked vs unspiked sample – reduction of +ve signal for true positives.
- Assay for the assessment of the neutralizing capacity of antibodies.
- Assays for measuring the level of the product and for assessing clinical relevance to products e.g., assays for relevant biomarkers or PK are required to evaluate the clinical impact of induced antibodies if these are detected.
- In some cases, cross-reactivity studies with other products based on the same protein.



# Screening assays

- First step in immunogenicity evaluation (mainstay)
  - Sensitive & capable of detecting all clinically relevant antibodies induced against the product
- Several platforms and formats/detection systems.
  - All detect antigen-antibody interaction but differ in their scientific principles.
  - Moderate throughput and automated
  - Relative merits and weaknesses need to be considered when developing/selecting an assay for use.
  - Attention to conjugation procedures, reagents such that detection is not compromised.

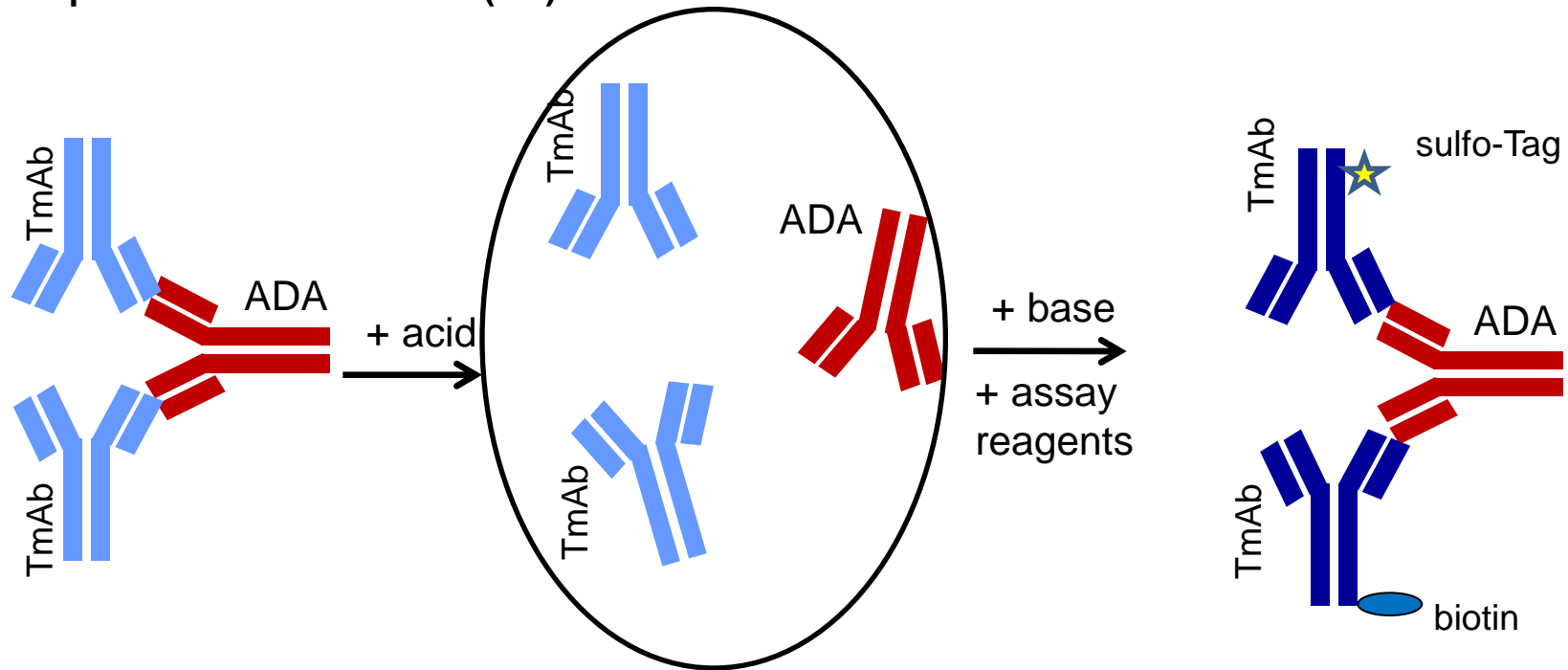
# Immunogenicity testing : Some Considerations

- Pre-existing antibodies – if detected, investigate reactivity and implement strategy; problematical from bioanalytical, efficacy & safety perspective
- Matrix effects from substances in samples can cause false positive or negative results.
  - Examples - soluble target, Fc receptors, complement components or complement receptors, disease specific factors such as rheumatoid factors should be evaluated & corrective measures implemented on a case-by-case basis as appropriate.
- Suitability of chosen approach should be justified while considering limitations of the respective methods.
- Some products have long half-life or are given chronically at high doses so samples may contain high levels of therapeutic/immune complexes which can interfere with detection of induced abs.
  - This needs evaluation and an optimal strategy defined and built in to the assay.
  - Assays where drug tolerance exceeds the level of therapeutic in the samples.

Although a suitable positive control can be used, it does not reflect the situation with clinical samples (varying isotypes, affinities etc within/between patients over time).

# Problem of residual therapeutic

Principle of acid dissociation: (AD)



Acid dissociation or a variation to remove the therapeutic & prevent immune complex formation.

Other options e.g., sample dilution, wash-out samples or a combination of above approaches.

Inclusion of any of the measures must not compromise detection of antibodies

# Immunogenicity testing

- Determination of the neutralizing potential is essential and deviation needs a strong justification.
- Any sample containing NAbs against the therapeutic reduces or abolishes the bioactivity of a known amount of the therapeutic.
- Two types of assays –
  - Cell-based and Non-cell-based
  - Regardless of the format, the assay should reflect neutralizing capacity.
- MOA of the therapeutic will dictate the format to use.
  - Non-cell-based assay relevant when a therapeutic MAb acts by binding to a soluble ligand thereby blocking it from interacting with its receptor thus inhibiting the ligand's biological action. Since the assay measures binding to the target and inhibition of binding activity if NAbs present, it reflects the MoA.
  - Cell-based assays recommended for MAbs where effector functions important for the clinical effect,

# Assay Controls and Reagents

- The identification and/or development of appropriate well characterized positive and negative controls is crucial. They are intimately associated with assay interpretation and distinguishing antibody positive from antibody negative samples.
- Positive antibody controls: for development, defining sensitivity, tolerance.
  - Polyclonal sera from hyperimmunised animals, affinity purified, mAbs, anti-idiotypic antibodies;
- Negative: for threshold/cut-off for 'discrimination' of antibody positive from antibody negative samples.
  - healthy sera, diseased sera, irrelevant antibody
- It is also very useful to prepare a panel of reference materials containing different amounts of antibodies and antibodies with different characteristics which can be used to characterize/validate assays and act as assay performance indicators.

# Interpretation of results

- Data interpretation - It is essential to establish clear criteria for deciding how
  - samples will be considered positive or negative, and also
  - how positive results will be confirmed.
- Harmonised way has been established for determining cut-point & also confirmatory cut-point
- Commonly, for a screening assay, a cut-off value which defines the assay threshold at or above which samples can be categorised as +ve is determined by using healthy/diseased sera and a statistical approach which purposely includes possibility of detecting false-positive results.
- Magnitude of response or titre evaluation to determine the level of antibodies is important.

# Reporting of Data

- Reporting should include
  - Rationale for choice of methods,
  - Sequence of testing,
  - Antibody incidence and the titre
  - Kinetics of response i.e., when onset initiated and the duration of response - transient/persistent, how long antibodies persist after treatment cessation.
  - Neutralizing capacity of the antibodies
  - Impact on PK, PD etc
  - Impact on Efficacy, Safety etc
- In certain circumstances, it may be feasible to further characterize the antibody response
  - Determine the isotype and IgG-subclasses
  - Determine cross-reactivity of the ADAs with relevant endogenous proteins
- Additional testing, e.g., antibodies for host cell proteins if significant amounts of HCPs present in product.

# Assays for comparative immunogenicity

- Comparative immunogenicity studies are always needed for biosimilars but rarely for a change of the manufacturing process of a given biological product.
- Immunogenicity testing of the biosimilar and the reference product should be conducted within the biosimilar comparability exercise by using the same assay format and sampling schedule ..... Analytical assays should be performed with both the reference and biosimilar molecule in parallel (in a blinded fashion) to measure the immune response against the product that was received by each patient.
- The analytical assays should preferably be capable of detecting antibodies against both the biosimilar and the reference molecule but should at least be able to detect all antibodies developed against the biosimilar molecule.

# Immunogenicity assessment

- Guideline does not recommend any particular assay
  - Evaluation of different platforms prior to final selection of screening assay; >1 assay platform may be needed for screening,
  - generic assay/strategy does not fit all; case-by-case approach needed
  - Assay choice and interference issues need to be clearly understood.
- Develop an understanding that a proper assessment is reliant on the study strategy, assays and execution of the assays.
- Advocates 'validated' methods for clinical evaluation
- For MAbs - General guideline + Mab guideline in conjunction for immunogenicity studies

Impact of antibody formation on clinical outcome is important and should be thoroughly evaluated.

ThankYou!

# Immunogenicity assessment of conjugated proteins and fusion proteins

- Elicitation of an antibody response with multiple specificities and variable affinity towards different epitopes is expected for novel biotherapeutic molecules such as engineered fusion proteins and chemically conjugated proteins.
- The evaluation of this response, in particular, the characterization of the specificity of the induced antibodies is challenging and may require multiple assays for measuring immune responses to various moieties.
- Alternatively, a strategy based on the competitive inhibition principle of the confirmatory assay to dissect the specificities of the antibodies to individual moieties can be used. For example, for a pegylated protein, the assessment strategy would comprise a screening assay using the pegylated therapeutic and testing of any positive samples using the whole therapeutic, the non-pegylated protein and the PEG moiety in a confirmatory assay.