Overview of EBF harmonisation activities for immunogenicity

Jo Goodman
on behalf of the EBF

EBF Autumn Focus Workshop

Today's challenges and solutions in assessing immunogenicity in patients

Altis Grand Hotel, Lisbon - 19th September 2018

EBF had limited activities focussed on immunogenicity prior to 2016

- Historically EBF activities around immunogenicity have been limited
  - Topic Team on the sense/non-sense of neutralising antibodies
  - Feedback as EBF during public consultation for guidance
  - Long-term stability testing for anti-drug antibody (ADA) assays

White Paper

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Bioanalysis

EBF recommendation for stability testing of anti-drug antibodies; lessons learned from anti-vaccine antibody stability studies

Long- and short-term stability testing of the analyte is one of the key parameters in bioanalytical method validation in support of pharmacokinetics. However, for immunogenicity testing, the scientific rationale for long- and short-term stability testing on quality control samples most often spiked with polyclonal antibody raised in a different species should be questioned. Therefore, the European Bioanalysis Forum (EBF) formed a Topic Team to discuss the scientific rationale for stability testing of anti-drug antibodies (ADAs). A review of EBF member companies' experience on ADA stability and on anti-vaccine antibodies from vaccine projects was the basis of this discussion. EBF recommends to perform short-term stability testing of the positive control, but not to perform long-term stability testing of ADAs in nonclinical and clinical studies.

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Background & scope

Testing for unwanted immunogenicity towards biological drugs is part of the ana-

in a manner that preserves antibody reactivity at the time of testing". It is further stated, that "Freezing and thawing patient sam-

EBF implemented an Immunogenicity Strategic Workstream (SW) in 2016

- Change from EBF Topic Team model to produce a tangible output in a short-time frame

- SW Membership:
  - David Egging - Synthon
  - Jo Goodman - MedImmune
  - Timo Piironen – Syrinx Bioanalytics
  - James Munday - Covance
  - Robert Nelson – Novimmune

- Culminated in Focus Workshop (FW) in September 2016
  - *"Current Analysis of Immunogenicity – Best Practices and Regulatory Hurdles"*
  - Focus on regulations, cut point and alternative approaches for neutralising antibody assays (NAb)
  - Slides: *http://www.e-b-f.eu/fw201609-slides/*

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Output of the first Immunogenicity FW

Conference Report

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Bioanalysis

Feedback from the European Bioanalysis Forum: focus workshop on current analysis of immunogenicity: best practices and regulatory hurdles

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Immunogenicity SW activities did not stop there

- One output from 2016 FW was standardising reporting of titre and drug tolerance
  - 2 Mini Workshops (mWS) at EBF Closed EBF Meetings
  - 1 mWS at EBF Open Symposium in Barcelona (2017)
  - Conference report has been written ready for publishing
- mWS on preclinical immunogenicity assessment, March 2017
- mWS at Closed EBF Strategy Meeting, March 2018
  - Ideas gathering amongst member company representatives leading to this FW
  - Main focus on positive controls and the value for stability assessments
- EBF Training Day on Critical Reagents with a dedicated session to immunogenicity, May 2018
  - Recommendations presented from team and mWS discussions
  - Slides: http://www.e-b-f.eu/td201805-slides
  - Presentation planned at EBF Open Symposium in Barcelona (2018)
  - Plans to publish a paper
- Involvement in AAPS-sponsored ADA Validation Testing and Reporting Harmonisation

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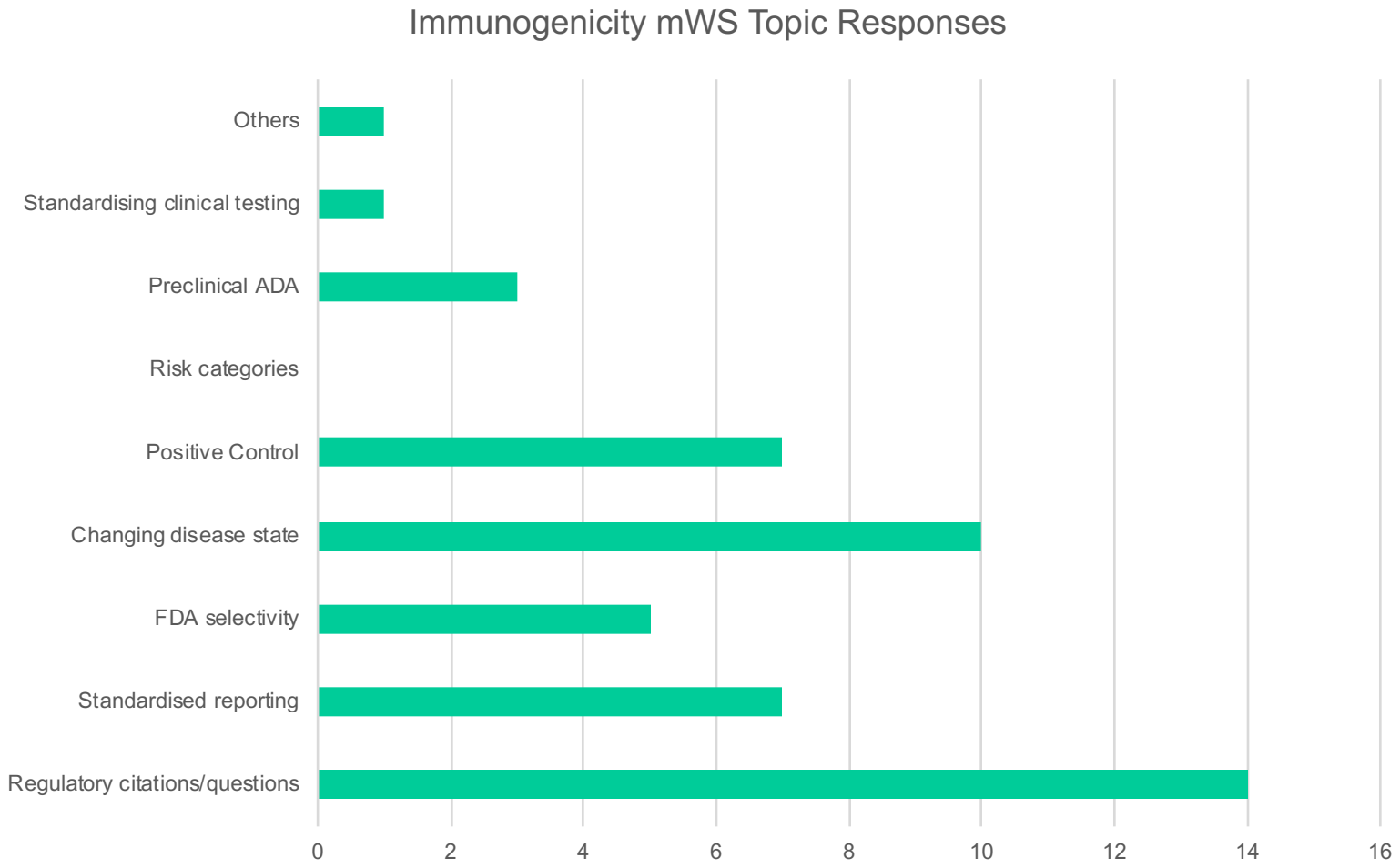
Immunogenicity topics popular within the EBF community (March 2018)



One extra suggestion: in all tiers are all validation parameters necessary?

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Minimal required dilution (MRD) and titre reporting

- Differing perspectives from EMA and FDA
  - EMA (2017)

real data (e.g. double background value) can be used to determine what will be considered the lowest positive result. For antibody positive samples, a titre needs to be determined using a standard approach and reporting the reciprocal of the highest dilution at which the sample gives a positive result. Another option is to report in mass units using a positive antibody control but this has caveats as explained above.

  - FDA (Draft 2016)

For patients who are confirmed to be ADA positive, determining antibody levels can be informative because it allows for the stratified assessment of ADA levels and their impact on safety and efficacy. These relationships may not be elucidated unless ADA levels are determined. Positive antibody responses may be reported as a titer (e.g., the reciprocal of the highest dilution that gives a readout at or just above the cut point of the assay), when appropriate. The MRD should be factored in the calculations of titers and provided when reporting titers. Reporting levels of antibodies in terms of titers is appropriate and generally understood by the medical community. Values may also be reported as amount of mass units of therapeutic protein product neutralized per volume serum with the caveat that these are arbitrary in vitro assay units and cannot be used to directly assess therapeutic protein product availability in vivo.

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So what is the challenge?

- Mixed approach for MRD mainly driven by differences in guidance

- If MRD is included should all dilution steps be included?
  - FDA only mentions MRD and interpretation of what constitutes MRD is different

1. *Only the reciprocal of the titre dilution is used*
2. *First sample dilution multiplied by the reciprocal of the titre dilution*
3. *All dilution steps within the method multiplied by the reciprocal of the titre dilution*
   - *First sample dilution*
   - *Acid dissociation*
   - *Dilution from methods such as SPEAD/ACE*
   - *Dilution from addition of labelled reagents*
   - *Any other source of adjustment*

- Prescribing physicians utilise titre as a way of comparing products

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Let's look at the numbers …..

- Bridging assay
- 1:10 sample dilution
- 2-fold dilution series for titre

Dilution Step #:	0	1	2	3	4	5
Reciprocal:	**1**	**2**	**4**	**8**	**16**	**32**
Reciprocal	1	2	4	8	16	32
Reciprocal * 1st sample dilution	10	20	40	80	16	32
If report using Log2	0	3.32	4.32	4.91	5.32	5.64
As above plus the labelled reagent dilution (1:3)	30	60	120	240	480	960
If the method also has acid dissociation	300	600	1200	2400	4800	9600

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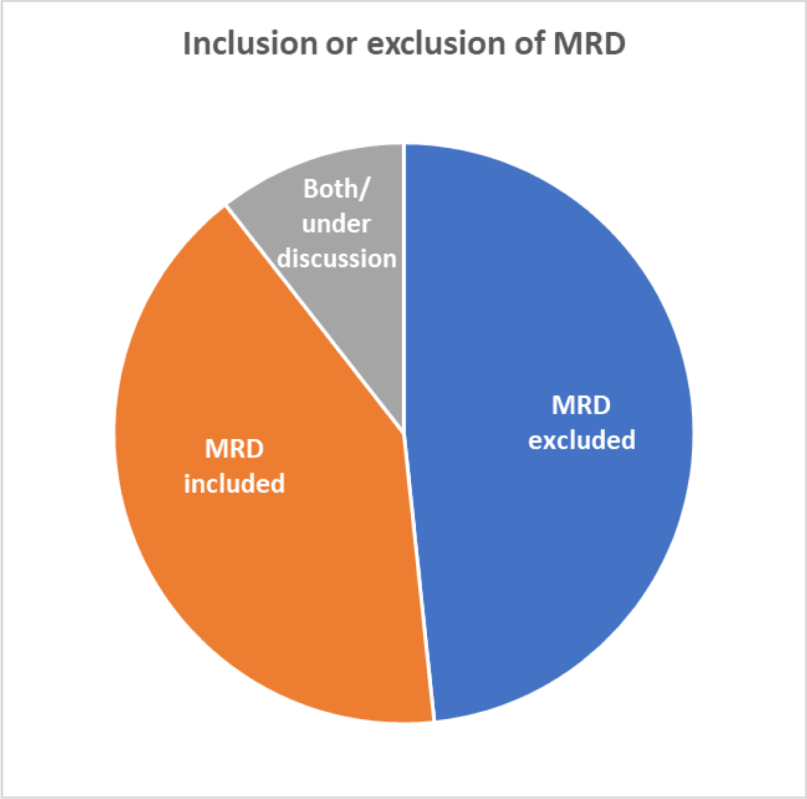
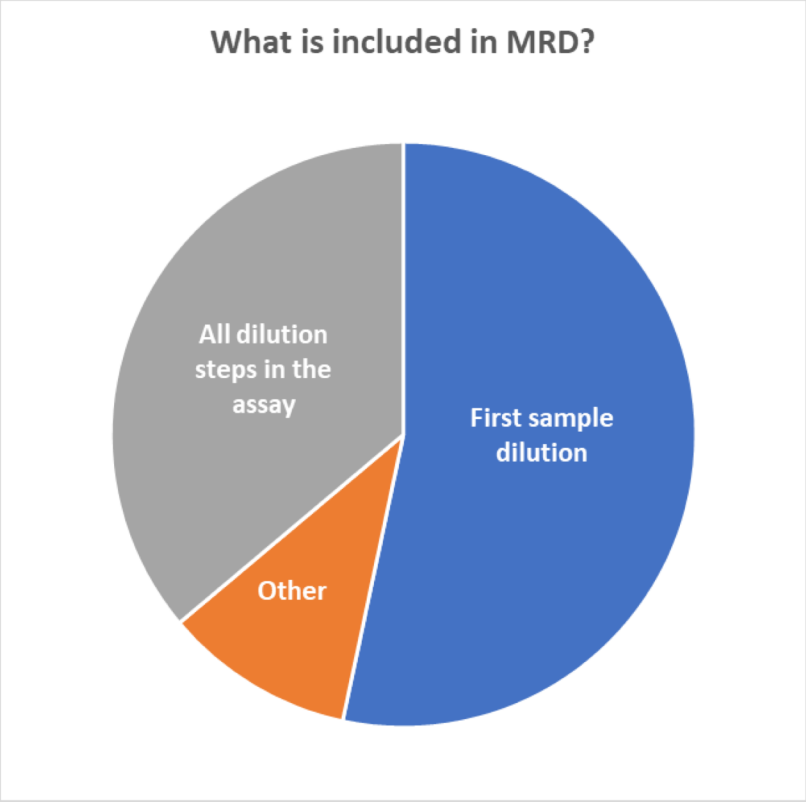
EBF survey results (2017) on MRD*

- 58 participants
- 62% Pharma, 36% CRO, 2% undisclosed
- 86% EU, remainder US and ROW





* EBF Open Symposium mWS 2017

No simple answer until guidance is aligned across agencies

- Approximately 2/3s in favour of standardising the approach, just under 1/3 not in favour, remainder in favour with reservations
- However, no consensus on was reached
- If all dilution steps are to be included, there are different challenges depending on the assay format
  - Acid dissociation
  - SPEAD/ACE
  - PandA
  - Gyrolab ADA mixing CDs
- Other approaches such as Signal Noise Ratio (SNR) that reports magnitude may be useful in some cases (Manning *et al.* (2017))
  - May be challenging for prescribing physicians to understand the label with this approach

- *Recommendation:* the approach taken should be clearly documented in the CSR and method documentation
- Guidance needs clearer language to remove ambiguity or allow flexibility in approach assuming adequate documentation

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Can drug tolerance approaches be standardised?

- ***"Highest concentration that does not interfere in the ADA detection method"*** **(Shankar *et al.* 2014)**

- Various methods can be utilised to help improve drug tolerance
  - Acid dissociation
  - Adjusting incubation times
  - Solid phase adsorption
  - Sample dilution
  - Sampling time points when drug has cleared
    - Challenge: EMA recommends that drug tolerance exceeds dosed levels yet mentions that fully tolerant assays may not always be possible
- EMA (2017) does not state how to perform but to address during method development
- FDA (Draft 2016) states that different amounts of purified ADA are added to ADA negative samples and adding different quantities of the protein therapeutic to those samples

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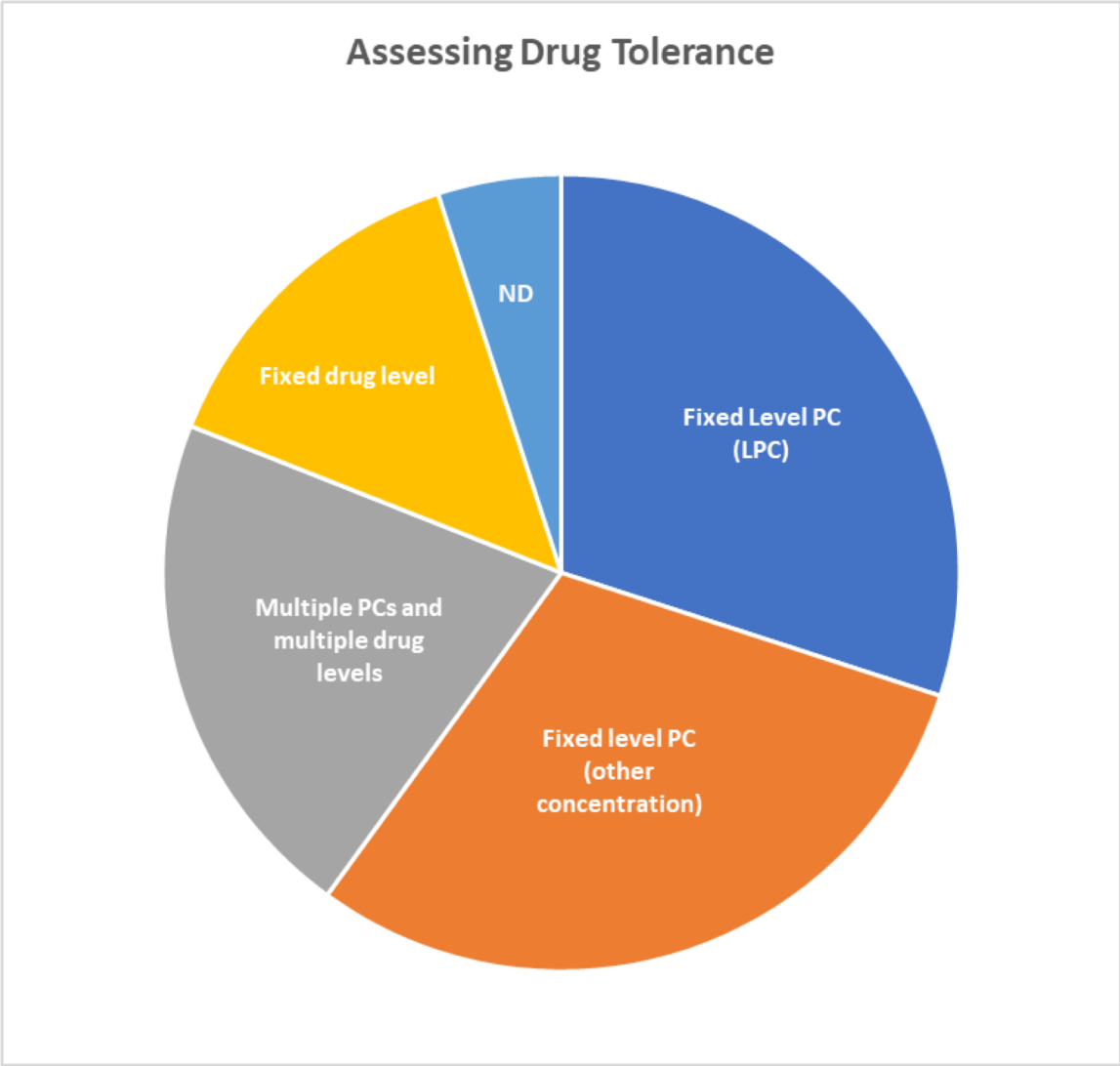
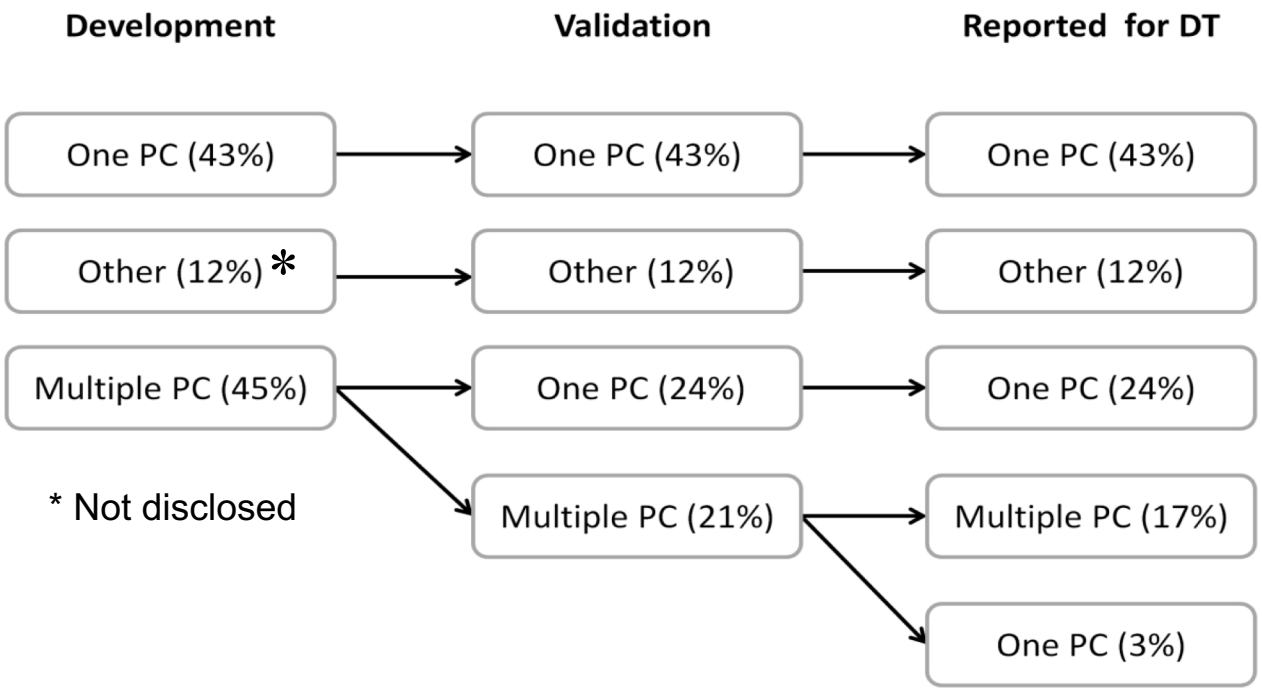
Assessing drug tolerance differs within the community



The majority report one positive control (PC) level



Considerations for the drug tolerance assessment

- Drug tolerance in validation is dependent on the surrogate positive control used
- This assessment is an estimation
  - In the real samples, responses may be detected in higher amounts of drug than those reported during validation
  - Useful in understanding potentially false negative results
- Relevance of assessing tolerance at the low positive control (LPC) for 'super sensitive' assays - assessing a higher level of ADA may be more appropriate
- Selection of the level(s) of drug to test may be best linked to expected concentrations in the samples
- Should the complex be frozen to reflect a sample?
- Standardisation on a common approach was not clear
- Expectation from agencies on the approach would be beneficial (collaborative discussion with industry)

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Positive control (PC) discussions

- Variety of approaches for positive controls
  - Monoclonals, polyclonals and/or panel of monoclonals
  - Usually what works best in the assay
  - Human controls from trials not generally used
- May switch PC when moving to neutralising antibody assays
- Range of immunisation protocols
  - Species, immunisation length, adjuvants, purification
  - Dependent on stage of project, time line, volume needed, company practice
- Selection of PC levels
  - MPC (value in validation and position)
  - Setting of LPC
  - Use of limits

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Stability discussions

- Relies on use of surrogate positive controls (PCs) that may not be reflective of human responses
- Spiking of the PC is testing the stability of that control and not the stability of samples
- Long-term stability (LTS) of antibodies is well documented in literature
  - E.g. Michaut *et al.* (2014), Pihl *et al.* (2014)
- Use of trending analysis for PCs and system suitability limits
- Utility of freeze-thaw (F/T) stability of surrogate PC
- No known failures within EBF community for LTS or F/T stability that was a stability issue
- Despite available data, approximately 1/2 of EBF community still perform LTS and almost all performing F/T stability

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EBF Immunogenicity SW activities going forward

- 2 sessions planned at Open Symposium in Barcelona, November 2018
- Immunogenicity session at EBF closed meeting in Barcelona in November 2018
- Critical reagents team working on a recommendation paper
- Decision tree for preclinical immunogenicity assessment

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Summary

- EBF formed an Immunogenicity Strategic Workstream in 2016
  - Focus Workshop (FW) in 2016
  - Conference paper report published in Bioanalysis
  - Discussions continue within the EBF core companies
    - Preclinical immunogenicity
    - Minimal required dilution and titre reporting
    - Drug tolerance
    - Positive controls
    - Stability
    - Critical reagents
- 2nd Immunogenicity FW
- EBF will continue to work on immunogenicity focussed topics presenting/publishing outputs

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Acknowledgements

- Immunogenicity SW members
- Michaela Golob
- Barry van der Strate
- Philip Timmerman

Questions

Drug tolerance – EMA (2017)

should be justified, especially if there are limitations of the respective methods. Additionally, residual therapeutic protein (drug) present in patients' blood can complex with induced antibody and hence reduce the amount of antibody detectable by assays. This interference may affect assays differently, depending on the assay format or type and the antibody characteristics and should be addressed during method validation. If such drug interference occurs, it may be circumvented/resolved by using a number of approaches e.g. by dissociating the immune-complexes with acid, removing excess drug by solid-phase adsorption, use of long incubation times and/or using an assay which allows sufficient sample dilution to avoid this problem. Sometimes, it may be possible to remove drug or target from the sample using lectins or xenogeneic antibodies. Such approaches must themselves be validated for effectiveness and shown to have no negative impact on analysis results. In some cases, the interference from residual therapeutic protein can be overcome by appropriate spacing of the timing between administration of product and sampling for antibody assessment i.e. allowing time for the product to be cleared from the circulation before sampling. This latter approach must not compromise the detection of antibodies or the treatment of the patient. In any case, the Applicant has to demonstrate that the tolerance of the assay to the therapeutic exceeds the levels of the therapeutic protein in the samples for ADA testing. Due to technical limitations it may not be always possible to develop fully tolerant assays. If this occurs, the best possible assay should be employed and the approach taken should be properly justified.

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Drug tolerance – FDA (Draft 2016)

tolerance early in assay development. The sponsor may examine drug tolerance by deliberately adding different known amounts of purified ADA into individual ADA-negative control samples in the absence or presence of different quantities of the therapeutic protein product under consideration and determining quantitatively whether the therapeutic protein product interferes with ADA detection. Results obtained in the absence and presence of different quantities of the therapeutic protein product under consideration should be compared. There should be a relationship between the quantity of antibody and the amount of therapeutic protein product required for a specified degree of inhibition. Data from pharmacokinetic studies may be useful in establishing optimal sample collection times. Acid dissociation pretreatment or other approaches may be used to disrupt circulating ADA-drug complexes, which may lead to increased assay drug tolerance. Interference from the therapeutic protein product can be minimized if the sponsor collects patient samples at a time when the therapeutic protein product has decayed to a level where it does not interfere with assay results.