European Medicines Agency

SUBMISSION OF COMMENTS ON GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

EMEA/CHMP/BMWP/14327/2006

COMMENTS FROM The Biotechnology Industry Organization/Sara Radcliffe, Vice President, Science and Regulatory Affairs

GENERAL COMMENTS

The Biotechnology Industry Organization (BIO) submits these comments on the European Medicines Agency's (EMEA's) draft guideline on *Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins*. BIO represents more than 1,100 biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and 31 other nations. BIO members are involved in the research and development of healthcare, agricultural, industrial and environmental biotechnology products. Our members invest heavily in the research and development of biotechnology and pharmaceutical products in the European Union (EU) and elsewhere, and employ thousands of highly skilled persons in the EU. We appreciate the opportunity to submit comments on the draft guideline.

Our comments have one common theme – the use of a risk-based assessment strategy for decisions related to immunogenicity assessment. The risk-based strategy has been embraced by the US regulators and the pharmaceutical industry. We would appreciate your consideration of inclusion of this strategy in this document.

We also recommend that the format of this document be made consistent with the "Requirements for First-in-Man Clinical Trials for Potential High-Risk Medicinal Products" and "Comparability of Biotechnology-Derived Medicinal Products" EMEA draft guidance documents.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 85 95
E-mail: mail@emea.eu.int http://www.emea.eu.int

SPECIFIC COMMENTS ON TEXT		
GUIDELINE SECTION TITLE		
Line no[1]. + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)**
General Comment	**Document Format Suggestions:** The Executive Summary and Introduction should summarize the content or intentions of the guideline document. The Introduction should contain brief background information with references for the reader to acquire more detailed information regarding concerns about immunogenicity of biotherapeutics and factors that may influence immunogenicity. Instead of a detailed review of immunogenicity, the Introduction should lay the ground work for the importance and value of a risk-based strategy for anti-drug antibody testing and characterization. The Main Text of the document should describe the expectations or recommendations for testing and characterizing for anti-drug antibodies, and when such testing/characterizing is appropriate based upon a risk-based strategy. We recommend that the term "animal models" be removed throughout the document and replaced with "nonclinical studies".	
Page 3, Executive Summary	The Executive Summary and Introduction do not summarize the content or intentions of the guideline document.	We suggest the following wording, "This Guideline is intended to provide guidance on nonclinical and clinical immunogenicity testing for biological therapeutics." "This document contains background information concerning the potential causes and impacts of immunogenicity and provides recommendations for performance of immunogenicity assessment utilizing a risk-based strategy …"

[1] Where available

Page 3, Executive Summary 3rd paragraph	The Executive Summary and Introduction sections describe expectations for clinical studies. The 3rd paragraph comment regarding the predictive value of animal models could be interpreted as suggesting that nonclinical immunogenicity assessment is not necessary for safety assessment. Although nonclinical immunogenicity assessment may not be predictive of human immunogenicity, it is important for evaluation of the drug exposure and the monitoring for ADA-related adverse events in toxicology studies. For these reasons, the guidance document should include the minimum expectations for assessment of immunogenicity in nonclinical studies and its purpose (i.e. screening for ADA is recommended, characterization of the ADA may be performed on a case-by-case basis as warranted by risk assessment)	We suggest inclusion of the following text, "Antibody generation to a biotechnology derived therapeutic protein in the nonclinical setting may have relevance related to adequate drug exposure and support the evaluation of adverse events. Thus, evaluation of immunogenicity in nonclinical studies is necessary to assist in the interpretation of the regulatory toxicological data." "Screening for ADA is recommended. The characterization of the ADA may be performed on a case-by-case basis as warranted by risk assessment."
Page 3 Executive Summary 3rd Paragraph	The Executive Summary 3rd paragraph contains information that is more suitable for the Introduction and should contain more specific information with references as a resource for the reader.	We suggest moving this paragraph to the Introduction section and the alternate wording "Although animal studies have been used to predict the relative immunogenicity of different proteins or protein analogs (Zwickl et al, others), they tend to inconsistently predict the incidence of human immunogenicity of biopharmaceuticals. Adverse events due to immunogenicity are rare in toxicology studies, but when they occur, they can be illustrative of potential adverse clinical events (e.g. TPO). The characterization of immunogenicity responses in nonclinical studies is performed primarily to address the concern of altered pharmacokinetics due to the presence of ADA that can bind to or clear the drug from the system and therefore alter the drug exposure level. In the clinical setting….."

Page 3 Executive Summary 4th and 5th Paragraph	Executive Summary The terms "standardised" and "standardisation" need clarification relative to usage in related efforts. These terms have been used in circumstances for evaluation of immunogenicity testing conducted by multiple companies for a similar cytokine replacement. However in the context of this document, the therapeutic compound is unique and the term "standardisation" is not relevant. Additionally, the word "standardized" in paragraph 5 appears to contradict the next portion of the sentence which states "adapted for each product on a case-by-case basis and taking a risk based approach". How can it be standardized and adapted on a case-by case basis? Note this word appears spelled different ways (European vs. American spellings).	We suggest removing the word standardize(d) unless it is better defined as to the intent. Or we suggest clarifying: "For a given product, sampling should be standardized across studies. The sampling schedule for each product is determined on a case-by-case basis."
Page 3, Executive Summary 4th Paragraph	Paragraph 4 is not appropriate for the Executive Summary	We suggesting omitting the 4th Paragraph, or moving it to section 4.5 Clinical Safety.
Page 3, Introduction 1st Paragraph	Rewrite: 1st Paragraph:	We suggest the alternate wording, "Most biological/biotechnology-derived proteins produce an immune response that is triggered by more than one single factor. This immunological response is complex and, in addition to antibody formation, other events such as T cell activation or innate immune response activation (inflammation) could contribute to potentially adverse responses. In practice, toxicologists monitor immunogenicity in nonclinical studies to evaluate the effects of anti-drug antibodies on drug exposure and to determine whether any adverse events observed in the toxicology studies could be associated with an untoward immune response."

Page 3, Introduction General Comment	The Introduction in this guidance provides a satisfactory background of information for why immunogenicity assessment is important, although references are needed to provide the reader with a resource for more detail. Because the Introduction adequately explains the need for immunogenicity assessment, the additional information in the Main Text section 4.1 is not necessary, unless it can incorporate the risk-based strategy (please see comment for Main Text Section 4.1)	
Page 3, Introduction 1st paragraph	The guideline document suggests a need to broadly characterize the "humoral and cellular immune response" induced by biotechnology-derived therapeutics. Is this document intended to provide guidance on assessing anti-drug antibody formation or is the intended scope more broadly related to the immune system? As written, the guidance is heavily weighted to anti-drug antibody assessment. The "cellular immune response" is a much broader subject that may be more relevant to Immunotoxicology guidance documents.	If the scope of the document is anti-drug antibody, we suggest replacing "…immune response…" with "…antibody formation…" for clarity. If the scope of the document includes the general "immune response", the Executive Summary, Scope, and Main Text require additional rationale and specific recommendations.
Page 3, Section 1 Introduction, 3rd paragraph, sentence	Add "an individual" to the following sentence: "Patient-related factors that might predispose **an individual** to an immune response …."	We suggest the following wording, "Patient-related factors that might predispose an individual to an immune response include: underlying disease, genetic background, immune status, including immunomodulating therapy."

Page 3 **Scope** General comments	The scope does not appear to describe the intended content of the document. – The Scope should state whether this guideline is intended to support the existing regulatory documents (references) and/or includes additional expectations. – Scope should state if this guideline covers nonclinical as well as clinical studies. – Scope should state a focus on testing for anti-drug antibodies (humoral). Although mentioned in the document, the cellular aspect is mechanism-based and should be addressed separately as an immunotoxicology issue (reference current immunotoxicology guidances). – Does Scope include the design of Immunogenicity Studies? (not assays, but the nonclinical or clinical studies?) See pg 8 "Immunogenicity Assessment Strategy"	We suggest the following wording: "The principles adopted and explained in this document are intended to provide guidance for nonclinical and clinical assessment of immunogenicity as defined by the assessment of anti-drug antibodies." If the intent of the document is also to include guidelines on the design of immunogenicity studies, please include recommendations for when a specific nonclinical or clinical "immunogenicity study" is expected (e.g. comparability).
Page 3, Scope 1st paragraph, 2nd sentence	The statement "These proteins and polypeptides are produced from recombinant or non-recombinant-cell culture expression systems." This limits the scope of this document to **cell culture expressed** proteins and polypeptide. Was this the intent of the authors? Are synthesized peptides or vaccines not in scope?	We suggest the following wording "This applies to proteins, polypeptides, their derivatives and products of which they are components."
Main Text General Comments	All sections would be better supported with references and examples.	We suggest including references or references to examples.

Page 4, **Main Text** Section 4.1	Because the Introduction adequately explains the need for immunogenicity assessment, the additional information in the Main Text section 4.1 is not necessary, unless it can incorporate the risk-based strategy (please see comment for Main Text Section 4.1). If the background information was intended for risk-based assessment, there is a need to clarify and provide guidance for use of risk-based strategy. This section would benefit from the use of literature references with respect to a review of many of the factors and facets of immunogenicity; this may also shorten the text overall. A detailed listing is not appropriate for this type of guideline. We request a discussion of risk-based immunogenicity strategy similar to that described in the EMEA guideline on comparability of biotechnology-derived medicinal products.	We suggest changing the title of Section 4.1 to "Risk-based Strategy for Immunogenicity Assessment of Therapeutic Proteins" and addressing the following issues/questions: "recommendations for host antibody testing and characterization strategies for nonclinical and clinical studies based upon risk assessment of the drug and the study conditions in which the antibodies were generated… The premise of the risk assessment strategy is to consider the severity of consequences of the antibody response to a protein therapeutic. The critical factors are the origin of the product and the presence and biological function of its endogenous counterpart." (please see Koren et al. Recommendations for Strategies, in draft) In nonclinical studies ….what needs to be considered when determining the extent of the immunogenicity assessment? In clinical studies …. what needs to be considered? We recommend adding "It is important to use a risk-based approach in order to determine the type and level of immunogenicity testing needed for a particular biotechnology derived therapeutic protein. The risk-based approach considers probability of an immunogenic response as well as the severity. The probability considerations may include: patient population, genetic factors, single vs. chronic dosing, human vs. foreign proteins, route of administration, HSA free vs. HSA containing formulations, level of purity, amount of aggregates. Severity considerations may include: endogenous vs. nonendogenous version of product, unique biological activity vs. redundant biological activity, sole therapy vs. other therapies, life threatening vs. non-life threatening disease, chronic vs. end stage disease, reversible vs. non reversible adverse events, replacement therapy vs. non replacement therapy."

Page 4 **Main Text** **Section 4.1** 4.1.1 "Genetic factors" 1st paragraph	**If the Main Text Section 4.1 will be retained with its current content, the following comments apply:** 1. Suggest rewording Genetic Factors section , due to confusing sentence	1. We suggest the following wording "Due to potential genetic differences in patients with an endogenous protein defect (abnormal or no production of the endogenous protein), when a therapeutic protein is used for substitution therapy immunogenicity responses between patients can be highly variable." We suggest that references/examples be added into the document.
2nd bullet 1st sentence	2. First line under "Genetic factors…", correct typo: "If the therapeutic protein is used for substitution, reduced levels or even lack of **an** endogenous protein, **it can** influence immunological tolerance, since for these……."	2. "If the therapeutic protein is used for substitution, reduced levels or even lack of an endogenous protein, it can influence immunological tolerance, since for these patients the physiological antigen may represent a neo-antigen."
4th bullet? Disease related factors	3. Disease-related factors should include a statement that the immunogenicity of a product may be different depending upon the stage of the disease. For example, patients with advanced cancer that receive heavy and frequent doses of chemotherapy are less likely to develop an immune response to a product as compared to cancer patients that are at a relatively earlier stage of the disease receiving therapies that may be less immunosuppressive.	3. We suggest deleting: "For some products, it has been reported that the susceptibility to an antibody response can be different for different indications. Therefore, immunogenicity may need to be studied separately for each disease." We suggest the alternate wording "For some products, it has been reported that the development of an antibody response can be different for different indications or different stages of the disease. Therefore, immunogenicity may need to be studied separately for each disease or stage of the disease."

Page 5 Section 4.1.2 2nd paragraph 2nd sentence	Protein related risk factors of immunogenicity *Protein Structure* Concern regarding the statement "Fusion proteins composed of a foreign and self-protein are of particular concern because of the potential of the foreign moiety to provoke an immune response to self-protein (antigen spreading). Identification of the antigenic site is advisable". *Comment/Rationale:* Identification of the site does not provide any utility unless such information will be used to potentially modify the molecule to reduce immunogenicity. A risk-based approach should be utilized for this effort.	We suggest the following wording "Fusion proteins composed of a foreign and self-protein are of particular concern because of the potential of the foreign moiety to provoke an immune response to self-protein epitope spreading. In the event that antibodies are generated to the drug product with corresponding impact on efficacy or safety, identification of the antigenic binding site specificity of the ADA should be considered using a risk-based approach"
Page 5 Section 4.1.2 2nd paragraph 6th sentence	Protein related risk factors of immunogenicity *Protein Structure* The final sentences in the "Protein Structure" section states *"*When the same protein is manufactured under different conditions there might be changes in pattern of post-translational modifications. *Consequently, antibodies induced by one product may or may not cross-react with another product.* It is also important to consider this aspect for immunogenicity testing*"*. It is not clear what guidance is being given here and further clarification would be helpful. Is this a concern for assay development, i.e. positive controls not cross react with the drug product from an alternate manufacturing process? Product-to-product ADA cross-reactivity?	Please clarify what is meant by the sentence "Consequently, antibodies induced by one product may or may not cross-react with another product."
Page 5 Section 4.1.2 3rd Paragraph 3rd & 4th sentences	Formulation Regarding the statement "Therefore, critical properties of excipients should be identified and characterized. The stability of the formulation and the composition and the source of excipients may alter immunogenicity…" The meaning of "critical properties" is not clear.	We suggest the alternate wording: "Therefore, the source and composition of excipients should be identified and the stability of the formulation determined. These factors should be considered as potential impacts upon the immunogenicity of the formulated product"

Page 5 Section 4.1.2 4th paragraph 1st sentence	Formulation Concern with wording in italics: *"*Impact of the condition of clinical use e.g. dilution in infusion solutions, use of diverse immediate containers, infusion devices of different material could *be the link to increased immunogenicity."*	We suggest an expanded sentence to read *"*The impact of the condition of clinical use, changes in formulation and/or delivery methods (e.g. dilution in infusion solutions, use of diverse immediate containers, infusion devices of different material) could also influence the immunogenic potential of a therapeutic protein."
Page 6 Section 4.1.2 5th Paragraph	Aggregation Include Adducts in title for "Aggregation" and within paragraph. Omit: "Aggregation of proteins may either reveal new epitopes or leads to the formation of multivalent epitopes, which may stimulate the immune system. Factors, which could be considered to contribute to aggregate formation, include formulation, purification processes, viral inactivation procedures and storage conditions of intermediates and finished product. The use of other proteins e.g. albumin as excipient may lead to the formation f more immunogenic aggregates. It is important to monitor the aggregate content of a product throughout its shelf life." Note: In second to last sentence of this paragraph. Correct typo: "*of"* not "*f*".	We suggest expanding the title to read "Aggregation and Adduct Formation" We suggest the alternate wording : "Aggregation of proteins or adduct formation with formulation excipients may either reveal new epitopes or lead to the formation of multivalent epitopes, which may induce an immune response. Factors which could be considered to contribute to aggregate or adduct formation include formulation, purification processes, viral inactivation procedures and storage conditions of intermediates and finished product. The use of, or lack of, albumin as an excipient may lead to the formation of more immunogenic aggregates or adducts. It is important to monitor the aggregate or adduct content of a product throughout its shelf life." For example according to Johnson & Johnson PRCA case reports on EPREX®, in non US markets, a key factor playing a primary role in cases of PRCA was the replacement of human serum albumin with polysorbate stabilizers. Prior to that the incidence of immunogenicity was very low.
Page 6 Section 4.1.2 6th paragraph	Excipients and impurities This paragraph although informative, does not appear to belong in this section. It is under section on excipients and impurities but discusses how various factors may impact immunogenicity and that a "suitable risk management strategy should be devised". Also, excipients and impurities are mentioned in the Formulation section.	We suggest the following options: Option #1: Move the excipients information from the Formulation section to the Excipients sections, Option #2: Combine Formulation and Excipients as one section.

Page 6 Section 4.2 Predictivity	Predictivity of nonclinical models The important message regarding nonclinical studies should be that immunogenicity assessment in nonclinical species is not intended for the purpose of predicting immunogenicity in humans, but rather to support the interpretation of the nonclinical study. Additional information as to how the immunogenicity information is used in conjunction with other nonclinical safety parameters should be included.	We suggest the alternate title "Nonclinical Immunogenicity Assessment" We suggest the alternate wording "In nonclinical studies it is not uncommon to obtain a relatively high incidence and level of antibodies especially to human, humanized or chimeric proteins and peptides. Such antibodies may affect pharmacokinetics, pharmacodynamics and bioavailability of the product in toxicology studies and this may result in altered drug exposure. Testing for binding antibodies should be performed. Some characterization as to confirmation and level of antibodies (titer or concentration) may be recommended for interpretation of the nonclinical study." "Evaluation of the neutralizing/clearing activity may be based on the assessment of PK/PD or biomarker data, if available. Determination of neutralizing antibodies in samples from nonclinical studies may be considered using the risk-based strategy on a case by case basis to provide additional information regarding whether animals were exposed to the active drug and the study provided and adequate assessment of potential toxicity. Depending on the type of drug and its mechanism of action, a competitive ligand binding assay or a bioassay may be appropriate."

Page 6 Section 4.2 Predictivity 1st and 2nd bullets	The bullet points describing the "other situations where immunogenicity studies" should be considered. The first two bullets of this section deal with manufacturing/ comparability issues relative to immunogenicity and should be combined into one bullet. The guideline implies that immunogenicity studies are the default requirement when a manufacturing change occurs, and exceptions must then be justified. This goes beyond the recommendations made in the EMEA guideline on Comparability of Biotechnology-Derived Medicinal Products, which bases the decision for further studies, such as immunogenicity, on a risk basis with such factors considered as complexity of the drug molecule, mode of action, posology, previous data, etc. (p. 4). The use of non-human species for comparing the immunogenicity of reference and comparator products can provide data that are difficult to interpret. Meaningful interpretation would require large numbers of animals to reveal statistically significant differences in the immunogenic potential of the two products undergoing comparison. Moreover, as the draft guideline currently acknowledges, even if differences are seen they may have limited predictive value for potential immunogenicity differences in humans. Such an approach may be useful for high-risk products that bear structural homology with an endogenous protein expressed by the selected nonclinical species. If statistically significant differences in immunogenicity in nonclinical species are observed in a comparability exercise, they should not be ignored. While this will not predict human immunogenicity, they do indicate that the product is not comparable. A risk-based approach should be used. Route of administration does affect immunogenicity but should not be related to comparability of products. SC and IM routes are recognized as more immunogenic routes of administration as compared to IV.	We suggested removing the following "There are some other situations where immunogenicity studies in animal models should be considered"and replacing it with "There are other situations where nonclinical immunogenicity studies should be considered using a risk-based approach." We suggest combining bullets 1 and 2 by deleting "In the development of the production process, formulation and route of administration, studies in animal models may aid in reducing the potential for immunogenicity" and replacing with "Although not predictive in humans, comparability studies in nonclinical species may be useful in evaluating the potential immunogenicity of therapeutic proteins undergoing changes in the production process or formulation. During the comparability exercise, the anti-drug antibody response induced by the reference standard is compared to the comparator product. A clear difference in the immunogenicity response in the nonclinical species would indicate that the products are not comparable." We recommend this document should mention the importance of considering manufacturing changes and comparability, with a reference to existing comparability documents (ICH Q5E) for details on those considerations rather than trying to recreate the considerations in this document (ICH Q5E, Step 4, Comparability of Biotechnological/Biological Products; Note for Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process (CPMP/ICH/5721/03, 1st December 2004): Section 2.5 Nonclinical and Clinical Considerations, EMEA guideline on Comparability of Biotechnology-Derived Medicinal Products)

Page 6 Section 4.2 3rd bullet, 3rd sentence	"Both humoral and cellular response should be considered." The 3rd bullet (3rd sentence) speaks to the need to consider cellular immune responses in addition to humoral immune (antibody) responses and appears to link the evaluation of the cellular response to high risk products where anti-drug antibodies have the potential to induce autoimmune reactions to endogenous proteins. However, no guidance is provided in terms of how cellular immunogenicity should be evaluated. There currently is no accepted industry or regulatory standard for conducting such an analysis and the additional value that assessment of the cellular immune response would provide is not clear. As stated in Annex 1: In most cases, development of a mature IgG response implies underlying antigen-specific helper T-cell involvement.	We suggest the following wording: "The humoral response to a therapeutic protein should be evaluated. Cellular responses may be evaluated on a case-by-case risk basis." Please clarify what is meant by "cellular immune response" and how to assess cellular responses.
Page 6 Section 4.2 Predictivity Last sentence	Predictivity of nonclinical models We suggest rewording of the statement *"Evolving in vitro and in vivo technologies e.g. transgenic mouse models may be useful for evaluating the potential immunogenicity of a given product."* Although these models may provide some information, until a mouse has identical MHC as well as all other components of the human immune repertoire, the only true test for immunogenicity is to go into a human. Thus, the use of these models has limited value.	We suggest the following wording: "Evolving *in vitro*, *in vivo*, and *in silico* technologies (e.g. T-cell activation, transgenic mouse models, and epitope mapping) may be useful for comparing the potential immunogenicity between products, however, they are not predictive of the human immunogenicity response."
Page 6-7 Section 4.3 Humoral & Cellular Assays	The title for section 4.3 "Development of Assays for humoral and cellular immune response" does not seem appropriate because the majority of the section deals with assay strategies for anti-drug antibodies. Furthermore, the section provides no guidance regarding assays for cellular immune responses other than to say that one should consider such.	We suggest the alternate title "Assay strategy for clinical assessment of anti-drug antibodies"

Page 6-7 Section 4.3 Title	The title "Development of assays for humoral and cellular immune response"	We suggest the alternate title " Clinical Immunogenicity Assessment"
Page 6-7 Section 4.3 1st paragraph	Discussions concerning Humoral and Cellular Assays should not be combined together into one section. The evaluation of humoral and cellular responses for immunogenicity is not consistent with current AAPS white paper strategies for immunogenicity. There are no accepted industry or regulatory standards or guidance documents available for how cellular immunogenicity assessment should be performed or interpreted. It is difficult to evaluate cell mediated responses. The assays require whole blood, are labor intensive, and have high backgrounds with extreme variability between individuals. Sample shipping from multi-center trial sites to laboratories would jeopardize the sample integrity. All of these factors would make the interpretation of the cellular response data difficult.	We suggest deletion of the 1st paragraph of section 4.3 and replacing it with: "It is important to select and/or develop the appropriate immunogenicity assay strategy and assays for each therapeutic protein using a risk-based assessment. The humoral response to a therapeutic protein should be evaluated. This is typically anti-drug antibody assays. Cellular immune responses and the feasibility of the performance of cellular immunogenicity assays may be evaluated as warranted by risk." We suggest clarifying what is meant by "cellular immune response" and how to assess cellular responses.
Page 7 Section 4.3 Assay Strategy 2nd paragraph	<u>Assay strategy</u> The strategies for nonclinical and clinical immunogenicity assessment may be different based upon risk assessment. Therefore, include a nonclinical strategy in the "Predictivity of non-clinical models" (or retitled "Nonclinical Immunogenicity Assessment") section. (See comments for Page 6, Section 4.2, Predictivity) We recommend a reference to the AAPS white papers by Mire-Sluis et al, 2004 and by Koren, et al. (in draft) and/or the Strategies publication by Shankar, et al. 2007.	We suggest the following wording for clinical assay strategy "In clinical studies, it is important to collect appropriate data regarding the appearance and characteristics of antibodies induced over time and assess how these findings may be associated with clinical outcomes. In general, samples should be screened for anti-drug antibodies using a binding assay, screen positives are confirmed in a confirmatory assay and then further characterized for titer or concentration. Samples that are confirmed positive should be further characterized for neutralizing antibodies." We suggest a decision flow chart for strategy: *See attached decision flow chart at the end of this document.*

Page 7 Section 4.3 *"Assay Strategy"* 2nd sentence	The second sentence describes the neutralization assay as a functional bioassay without the allowance for other formats such as ligand binding assays. The neutralization assay may be a cell-based or a non-cell based assay depending upon the complexity of the biology induced by the drug and the ability to develop a cell-based assay of acceptable sensitivity and specificity. A recently published white paper on NAb assays (Gupta et al. 2007) provides further details on situations where cell-based assays are irreplaceable for NAb detection and examples when these assays are difficult to develop due to various confounding factors warranting the need to develop a non cell-based NAb assay. Competitive ligand binding assays can be a viable option early in programs (Phase 1, Phase II) and/or depending upon mode of action of drug throughout a program (all phases). Additionally, in many cases competitive ligand assays may be more sensitive than a bioassay and hence provide more information than a bioassay. Correct typo in last sentence: change "were" to "where"	We suggest changing the sentence on functional bioassays from "…functional bioassay(s) for the assessment of neutralizing capacity" to: "assays for the assessment of neutralizing antibodies. These assays may be competitive ligand binding assays or bioassays depending on mode of action of drug, risk assessment evaluation, and testing strategies." "A neutralization assay should be performed on confirmed antibody positive samples for the detection and characterization of neutralizing antibodies. Functional bioassays are recommended and especially important for high risk products that bear resemblance to a non-redundant endogenous protein. There are situations where it is difficult to develop a bioassay due to various confounding factors, such as the type of drug or the mechanism of action, warranting the need to develop a non cell-based assay."
Page 7 Section 4.3 Assay strategy 2nd paragraph	With regard to the statement: "for further details on the proposed strategy for antibody detection and characterisation see Annex 2": Annex 2 is confusing and the text in the Assay Strategy section does not support the specifics listed in the diagram. For example, why is "Surface plasmon Resonance Assay" listed as a specific technology? It is inconsistent with the rest of the "types of assays" categories: binding, confirmatory, bioassay. A decision tree process flow chart outlining the strategies might be more useful. References to the literature would also be useful e.g. Koren, et al. or Shankar, et al.. . Should Annex 2 be called Annex 1?	We suggest replacing the current Annex 2 diagram with a decision flow chart for screen – confirm - quantify – characterize (Nab, Isotype, Specificity…)

Page 7 Section 4.3	The "Types of antibody assays" in the assay strategy section describes the expectations of the assays and may be more appropriately titled "Expectations of Antibody Assays". Annex 1 also has a section titled "Types of Antibody Assays" which actually describes the types of assays.	We suggest replacing "Types of antibody assays" with "Expectations of Antibody Assays"
Section 4.3, sub-section entitled "*Screening assays",* 2nd sentence	This section must emphasize the importance of the assay cut point that represents the level of response in the screening assay below which a sample is negative for antibodies and above which a sample is suspected to contain antibodies and requires further confirmation in a specificity assay. The derivation and validation of the assay cut point are important activities during assay development and validation, respectively. Mire-Sluis et al. 2004 recommend using the mean + 1.645 SD where 1.645 is the 95th percentile of the normal distribution of the population of serum tested to derive the assay cut point value. A screening assay that is unable to detect any positives in a pre-clinical or clinical study casts suspicion on the ability of the assay to detect low positives.	We suggest omitting: "This implies that detection of some false positive results is inevitable as absolute screening-assay specificity is normally unattainable and false negative results must be avoided" and replacing it with the alternate language "A statistical approach should be used to determine the assay cut point to distinguish between antibody-negative and positive samples. False negative results must be avoided. Using a risk-based approach, it is more appropriate to have 5% false positives than false negatives."

Page 7 Section 4.3 5th paragraph Assays for specificity and confirmation	"It is usually advisable to use a different assay format that used for the screening assay". Though this approach may be applicable in some cases, in other cases this can lead to discrepant results between assays. Different assay technologies have their own advantages and disadvantages based on the scientific principles they employ; therefore, they are not inherently the same. For example, surface plasmon resonance technology may be capable of detecting low affinity antibodies in real-time, however, it may not be as sensitive as other assay formats. Similarly, a bridging ELISA assay may offer high specificity, however, may be prone to high drug interference. Moreover, cut point for each of these assays will need to be established separately based on the subject sera background values within each assay, which may or may not result in the same level of sensitivity for each assay. Since many of the important parameters, such as sensitivity, drug tolerance, ability to detect low affinity antibodies etc. will not be the same between assays, confirming the specificity of antibodies detected in a different assay may lead to discrepant results. Competition of screening antibody positive samples by incubation with excess drug and testing in the same assay keeps the assay formats as close as possible. Statistical methods, such as the "t-test," have been proven to be robust and appropriate for confirming the antibody positive samples.	We suggest the following wording: "It is advised that the assay to confirm the specificity of screening antibodies be selected carefully and statistically interpreted. A competition assay can be performed by incubating positive samples with excess drug, thereby keeping the assay format the same. In cases where a different assay format is chosen to confirm the specificity of detected antibodies, the interpretation of results should include consideration of the drug tolerance, cut points and sensitivity that may differ from the screening assay."

Page 7 Section 4.3 3rd bullet 1st sentence Neutralization assays	Neutralization assays. Same comments as Section 4.3 Assay strategy, above.	"assays for the assessment of neutralizing antibodies. These assays may be ligand binding assays or bioassays depending on mode of action of drug and risk assessment evaluation and testing strategies." "A neutralization assay should be performed on confirmed antibody positive samples for the detection and characterization of neutralizing antibodies. Functional bioassays are recommended and especially important for high risk products that bear resemblance to a non-redundant endogenous protein. There are situations where it is difficult to develop a bioassay due to various confounding factors, such as the type of drug or the mechanism of action, warranting the need to develop a non cell-based assay."
Page 7 Section 4.3 4th bullet 2nd sentence	Assay Validation *(this should be a header, not another bullet)* The "assay validation" section states that "Validation studies must be conducted to establish that assays show appropriate linear responses to relevant analytes …" Typically, definitions of linearity contained in regulatory guidance do not apply for anti-drug antibody assays which are quasi-quantitative due to the lack of availability of a valid reference standard, which is the case for most biological therapeutics. Thus, defining what is meant by linear responses may be useful. This sentence also uses the term "accuracy" as an assay validation parameter. The main analyte that immunogenicity assays can detect are antibodies that bind to the drug and since appropriate reference controls for the detected antibodies are generally not available, the term "recovery" should be used instead of "accuracy".	We suggest the following wording be changed "Validation studies must be conducted to establish that assays show appropriate linear responses to relevant analytes …" Please delete: "Assays need to be validated for their intended purpose. Validation studies must be conducted to establish that the assays show appropriately linear responses to relevant analytes as well as appropriate accuracy, precision, sensitivity, specificity and robustness." and replace with: "Assays need to be validated for their intended use. A suitable positive control antibody should be selected for this purpose. Validation studies must be conducted to establish that the assay shows a concentration-dependent response to the selected positive control antibody as well as appropriate precision, sensitivity, specificity, robustness and recovery." We suggest that a reference to Mire-Sluis, et al would be helpful.

Page 8 Section 4.3 Standardisation and reference materials 1st sentence	Standardization and reference materials We are concerned about the statement "Assays must be standardized….requires appropriate reference materials and the use of relevant biological standards. Reference materials and standards are essential for assay calibration and validation". Most novel biotherapeutics that are not recombinant forms of endogenous proteins do not have true standards or reference materials available.	We suggest adding the additional statement "Many novel biotherapeutics do not have true standards or reference materials. In this case, the use of appropriate positive controls is recommended."
Page 8 Section 4.3 Antibody Characteristics 1st paragraph	This appears to overlap with the introductory paragraph above it "Characterisation of Antibodies": "If antibodies are induced in patients, serum or plasma samples need to be characterised…."	We suggest moving this paragraph up to the introductory paragraph of "Characterisation of antibodies" with the following wording change. This allows each subsequent bullet to be covered by risk-assessment: "If antibodies are detected in patients undergoing therapy, a risk-based assessment should be performed to determine the need for additional antibody characterization and how the findings may be associated with clinical outcomes. Characterisation may include identification of antibody class and subclass (isotype), affinity, specificity, and/or neutralizing capacity."
Page 8 Section 4.3 Antibody Characteristics 2nd paragraph	The second paragraph is unclear as to intent. "Specificity" to the drug product is demonstrated in the confirmation assay. However, this paragraph is requesting a demonstration of specificity to the "active protein" distinguished from contaminant. Is the emphasis on identifying ADA to the active protein or characterising ADA to active protein and all contaminants? On a risk-basis, if a product is a conjugate or fusion protein, identification of the specificity of the ADA to a high-risk component of the product is advisable. However, an evaluation of the specificity of the antibodies to product-related and process-related components could be difficult and would require (a) the identification of these contaminants and (b) the availability of these substances in a purified form at sufficient quantities for routine use in confirmatory assays which could be challenging. If the contaminant is known and can be isolated, then the cross-reactivity of detected antibodies towards it should be studied using a risk-based approach.	"Although specificity to the therapeutic protein is demonstrated in the confirmation assay, further characterisation of specificity of anti-drug antibodies to the active protein component(s) of the drug may be considered for confirmed positive samples based on risk assessment."

Page 8 Section 4.3 Immunogenicity Assess Strategy	*Immunogenicity Assessment Strategy –design and interpretation*. This section may be out of place. Is it within the scope of this document? If so, this topic should be added to the scope. This topic may be more appropriate in the next section on Page 12, "Recommendations for routine monitoring…."	We suggest moving the "Immunogenicity Assessment Strategy paragraph to the "Recommendations for routine monitoring of changes in clinical response…" section.
Page 9 Section 4.4 1st paragraph Last sentence	<u>Potential Clinical consequences of immunogenicity</u> The last sentence states "the risk of immunogenicity needs to be considered individually for each indication/patient population." The term "risk" instead of "risk assessment" may be interpreted as the evaluation only of the probability of the development of anti-drug antibodies and not the clinical impact of the presence of the ADA. A therapeutic may have a high risk of causing antibody production, but the clinical impact of such a response could be negligible. Risk assessment needs to be defined early in the document.	We suggest changing the sentence to "Therefore, the risk assessment for immunogenicity needs to be considered individually for different indications and populations."
Page 9 Section 4.4 2nd paragraph *"Consequences on Efficacy",*	It may be more appropriate to place this discussion in the beginning of the guidance when the rationale for immunogenicity testing is discussed.	We suggest placing this discussion in the beginning of the guidance where the rationale for immunogenicity testing is discussed.
Page 9 Section 4.4 2nd paragraph *"Consequences on Efficacy",* *1st sentence*	1st sentence Other considerations may include: amount of antibodies, subclass of antibodies. This sentence would be more appropriately placed in the introductory paragraph above it, as it is applicable to all consequences mentioned, not just efficacy.	We suggest changing the first sentence to "Factors which influence whether antibodies to a therapeutic protein will induce clinical consequences may include the epitope recognized, the affinity, class and subclass as well as amount of antibodies generated." We suggest moving this to the 1st paragraph of this section.

Page 9 Section 4.4, 2nd paragraph *"Consequences on Efficacy",*	The text diminishes the importance of non-neutralizing antibody impact on efficacy. "Clearing" antibodies may be neutralizing or non-neutralizing, and are important in the discussion of efficacy due to their impact on pharmacokinetics. An additional consideration is "Binding", non-neutralizing, antibodies which may increase, rather than decrease, the efficacy of a product by prolonging the half-life, or stimulating a pathway or mechanism. The text would be more cohesive if the "Impact on pharmacokinetics…" paragraph in Section 4.5 was moved to section 4.4 and retitled "Consequences on Pharmacokinetics"	We suggest the following wording: "Antibodies recognising epitopes on the therapeutic protein not linked to activity are associated with fewer clinical consequences. However, such antibodies can influence pharmacokinetics and, as such, influence efficacy. "Clearing" antibodies may be neutralizing or non-neutralizing, and reduce efficacy by removing the therapeutic protein from circulation. Non-neutralizing, "binding", antibodies, may increase, rather than decrease, the efficacy of a product by prolonging the half-life, or stimulating a pathway or mechanism." Neutralizing antibodies may inactivate the drug with or without clearance. The loss of efficacy may be characterized through the Assay Strategy described in Section 4.3 as needed."
Page 9 Section 4.4, 2nd paragraph *"Consequences on Efficacy",* 5th sentence	"Discrimination between neutralizing and non-neutralizing antibodies is of great importance, and the assays used should be able to discriminate accordingly (see section 4.3)". A lack of efficacy may be detected through pharmacodynamic markers rather than through the development of neutralization assays. Nab assays may help to explain a loss of efficacy but should not be considered required as suggested by the statement. The statement "the assays used should be able to discriminate accordingly" suggests a need for a non-neutralizing positive control during Nab assay development. If this is the intent of the statement, there is insufficient information provided to understand how such an evaluation should be done and to what extent. It is often difficult to obtain a relevant non-neutralizing positive control antibody especially towards monoclonal antibody type drug products to test the NAb assay's ability to distinguish between neutralizing and non-neutralizing antibodies. Whenever a non-neutralizing positive control antibody is available this evaluation should be conducted as warranted by risk assessment.	We suggest the alternate wording: "Determination of neutralizing antibodies from confirmed screen positives, and the assays used, should be appropriate (see section 4.3)". Most importantly, the assay should be able to identify clinically relevant neutralizing antibodies."

Page 9 Section 4.4, 4th paragraph *"Consequences on Safety",* 1st Bullet *"Acute Consequences"*	The section on "acute consequences" linking antibodies to infusion reactions is misleading. Infusion reactions can commonly occur due to cytokine floods or product related toxicities that have nothing to do with antibodies to the drug product. A presentation by P. Keegan of the FDA in 2005 stated in a presentation that "the term infusion reaction has no regulatory definition". Furthermore, her slides state that "anaphylaxis appears to be a rare cause of infusion reactions" since "most infusion reactions are most common on initial exposure and less frequent/severe reactions observed on re-challenge." We recommend that this section be re-written as it applies to antibodies to the drug product and differentiate such from other causes of infusion associated reactions.	We suggest the following wording: "One of the clinical problems associated with treatment with biotechnology-derived therapeutic proteins is development of infusion reactions. Infusion reactions more frequently occur on initial exposure and less frequent/severe reactions are observed on re-exposure. Acute infusion reactions may occur within 24 hours and delayed ones may develop days after initiation of treatment and upon subsequent treatment. Acute reactions can be true allergic, namely IgE-mediated type I reactions (anaphylactic reactions), including hypotension, bronchospasm, wheezing and/or urticaria. However, the great majority of infusion reactions are characterized by more nonspecific symptoms and are often classified as anaphylactoid ones (i.e. probably nonallergic). A range of symptoms including headache, nausea, fever or chills, dizziness, flush, pruritus, and chest or back pain have been described in relation to infusions."
Page 10 Section 4.4, 7th paragraph *"Consequences on Safety",* 2nd Bullet *"Non-Acute Consequences"* *Autoimmunity*	<u>Autoimmunity</u> In the "autoimmunity section" please clarify that this pertains to therapeutics that have endogenous counterparts.	We suggest rewording the 1st sentence to: "Antibodies developed against therapeutic proteins with endogenous counterparts may cross react with the endogenous proteins in cases where endogenous protein is still produced (e.g., erythropoeitins)."
Page 10 Section 4.5 title	The title "Pre-authorization signal detection in clinical setting" is not clear.	We suggest defining what is meant by "Pre-authorization signal detection in clinical setting" or changing the header so the meaning is clear.

Page 10 Section 4.5, Clinical Safety 1st bullet *"Rationale for sampling schedule…",* 2nd paragraph	The draft guideline acknowledges that an antibody response could be transient or persistent but does not mention how this information should be used to determine the overall immunogenicity of a product.	We suggest adding the additional text after the current sentence 3 of this paragraph: "Both transient and persistent antibody responses should be considered when evaluating the clinical consequence of immunogenicity of a product. Although transient antibodies are less likely to have a clinical significance, the evaluation should be weighed with the assessment of risk."
Page 11 Section 4.5, Clinical Safety 1st bullet *"Rationale for sampling schedule…",* 3rd paragraph 3rd sentence	"Adequate follow-up of patients for measuring immune response after discontinuation of treatment should be implemented to evaluate immunogenicity in absence of the therapeutic protein." This statement does not allow for consideration of risk assessment.	We suggested the alternate wording: "Follow-up of patients for measuring immune response after discontinuation of treatment should be considered for evaluation of immunogenicity in absence of the therapeutic protein, when warranted by risk assessment."
Page 11 Section 4.5, Clinical Safety 2nd bullet *"Impact on pharmacokinetics of product"*	The content of this paragraph would be more appropriate with the "Potential clinical consequences" section. It is unclear why impact on pharmacokinetics is grouped with the surrounding text. Anti-product antibodies can affect the accurate measurement of product levels in typical PK immunoassays. It should be recommended that the PK assay be assessed for the effect of antibody interference on accuracy and precision and that results be interpreted appropriately if an immune response occurs.	The text would be more cohesive if the "Impact on pharmacokinetics…" paragraph in Section 4.5 was moved to section 4.4 and re-titled "Consequences on Pharmacokinetics" We suggest the additional text to the end of the paragraph: "To allow a meaningful interpretation, it is recommended that the effect of anti-drug antibodies on the performance of the PK assay be assessed. This may be achieved by evaluating the spike-recovery performance of the PK assay in the presence of the anti-drug positive control antibody. If anti-drug antibodies interfere with the ability of the PK assay to reliably detect drug levels, an alternate approach of measuring biologically active drug levels may be considered."

Page 11 Section 4.5, Clinical Safety 3rd bullet "*Methodology aspects to assess comparability of immunogenicity",* 3rd paragraph 3rd sentence	This section recommends that a product immunogenicity comparability study be conducted in humans – "Applicants should make an effort to select a homogeneous patient population that allows for such comparisons." This is probably beyond the scope of this document and the draft EMEA guidance on biocomparability sufficiently addresses biocomparability without recommending human studies. This guidance should be referenced.	Please refer to the EMEA guidance on biocomparability.
Page 12 Recommendations for routine monitoring of changes in clinical response …"	The content of the first seven paragraphs of this section would be more appropriately placed in the Immunogenicity Assessment Strategy section on Page 8 Section 4.3. There are many overlapping considerations.	We suggest incorporating the content of the first seven paragraphs into Section 4.3 Immunogenicity Assessment Strategy, including the Paediatric paragraphs.
Page 12 Section 4.6 2nd sentence	<u>Risk Management Plan</u> In section 4.6 the second sentence states "This should take into account risks identified during product development and potential risks." The sentence is worded in a confusing manner and should be reworded for clarity. .	We suggest the alternate wording for section 4.6 "Potential risks of a biotechnology product based upon mode of action or type of compound as well as nonclinical data, should be considered as part of the risk management plan submitted in accordance with EU legislation and pharmacovigilance guidelines."
Annex 1 Comments	General comment: The "types of antibody assays" information in the Annex 1 should be combined with the "Types of antibody assays" section in Section 4.3 page 7. There is not enough information under each of these classifications to warrant separating them and we are not clear as to the rationale for separating them.	We suggest moving the "Types of antibody assays" content in Annex 1 to the "Types of antibody assays" section in 4.3 page 7.

Page 14 Annex1 Screening Assays 1st & 2nd paragraphs	Concern regarding the statement: "Screening methods include immunoassays, radioimmunoprecipitation assays and surface plasmon resonance assays" RIP and SPR are "immunoassays" in different forms. Although screening assays are usually immunoassays, other formats are possible, such as chromatography.	We suggest the alternate wording of 1st and 2nd paragraphs combined: "Screening assay are primarily immunoassays, however other techniques may be used. Immunoassays are based on a variety of formats such as binding, bridging, capture (sandwich), and competitive, and they use a variety of detection systems including radiolig and enzymatic, fluorescent, chemiluminescent, or electrochemiluminescent labels. All procedures detect antigen-antibody interaction (binding), but may differ in their underlying scientific/technical principles."
Page 14 Annex1 2nd bullet Assays for dissecting specificity & confirming…	"Assays for dissecting specificity and confirming antibody positivity" is a long descriptive title; we, suggest shortening it to "Confirmatory Assays"	We suggest that the alternate title to the second bullet be "Confirmatory Assays", and move "Assays for dissecting specificity and confirming antibody positivity…" to the text.
Page 14 Annex1 2nd bullet Assays for dissecting specificity & confirming… 1st paragraph 2nd sentence	Concern regarding the statement: "to achieve confirmations of specificity it is advisable to select an assay based on a different scientific/technical rationale than that used for the screening assay" Same comments as Page 7, Section 4.3, 5th paragraph, "Assays for specificity and confirmation" The paragraph on competitive assays says "Analytical immunoassays such as immunoblotting … can be used to dissect the specificity", however this approach should be presented as not necessarily being needed for routine antibody detection. The paragraph should also note that there are perhaps additional characterisation techniques that may be used as warranted by the risk assessment.	We suggest the alternate wording: "Assays for confirming the specificity of screened positive samples should be selected and designed carefully and statistically interpreted. A competition assay can be performed by incubating positive samples with excess drug, thereby keeping the assay format the same as the screening assay. In cases where a different assay format is chosen to confirm the specificity of detected antibodies, the interpretation of results should include consideration of the drug tolerance, cut points and sensitivity that may differ from the screening assay." "Analytical immunoassays such as immunoblotting and surface plasmon resonance analysis can be used to further characterize the specificity of the detected antibodies if warranted by risk.

Page 14 Annex1 3rd bullet Neutralization assays	This section leads in with the assumption that the neutralization assay will be a bioassay, however, this is not always the case. *See comments in Section 4.3 Assay strategy.*	"These assays may be ligand binding assays or bioassays depending on mode of action of drug and risk assessment evaluation and testing strategies."
Page 14 Annex1 4th bullet Assays for Assessing cell-mediated immune responses	*Same comments as in Sections 4.2 and 4.3 on this topic.* There are no accepted industry or regulatory standards or guidance documents available for how cellular immunogenicity assessment should be performed or interpreted and none are included in this section. The examples listed are mechanistic, immunotoxicity evaluations. It is difficult to evaluate cell mediated responses. The assays require whole blood, are labor intensive, and have high backgrounds with extreme variability between individuals. Sample shipping from multi-center trial sites to laboratories would jeopardize the sample integrity. All of these factors would make the interpretation of the cellular response data difficult.	We suggest removing cellular immune response assays section from the Annex.
Page 15 Annex 1	*Assay Characteristics* *Standardisation* *Interpretation* Earlier comments and recommended rewording affect these sections of Annex 1	Update Annex 1, if earlier comments and suggested wording changes were accepted.
Page 17 Annex 2	Should Annex 2 be called Annex 1? It is the first Annex referenced.	

Page 17 Annex 2	The figure suggests using two different assays to screen all samples for binding antibodies. This approach will likely lead to discrepancies in incidence rates. The figure also recommends using surface plasmon resonance which may not be appropriate for all products. Different types of assay formats may be needed dependent upon the drug characteristics, dosing and patient population for use. The figure should indicate that (i) samples are termed positive only if they test positive in the confirmatory immunoassay, (ii) the bioassay is conducted on samples that test positive in the confirmatory immunoassay. The terms screening immunoassay, confirmatory assay and neutralization assay should be used in the flow diagram to indicate the 3-step testing strategy. A figure legend should be added to state that testing strategy should be based on risk and use appropriate assay formats for the product, dose and indication. Please see the attached figure.	We suggest replacing the current Annex 2 diagram with a decision flow chart for screen – confirm - quantify – characterize (Nab, Isotype, Specificity…) We suggest the insertion of a reference to Koren, et al. or Shankar, et al.

These comments and the identity of the sender will be published on the EMEA website unless a specific justified objection was received by EMEA.

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Koren, et al. (Submitted: Journal of Immunological Methods) Recommendations on Risk-Based Strategies for Detection and Characterization of Antibodies against Biotechnology Products

Figure 1. Step 1: Recommended testing strategy for detection of anti-drug antibodies

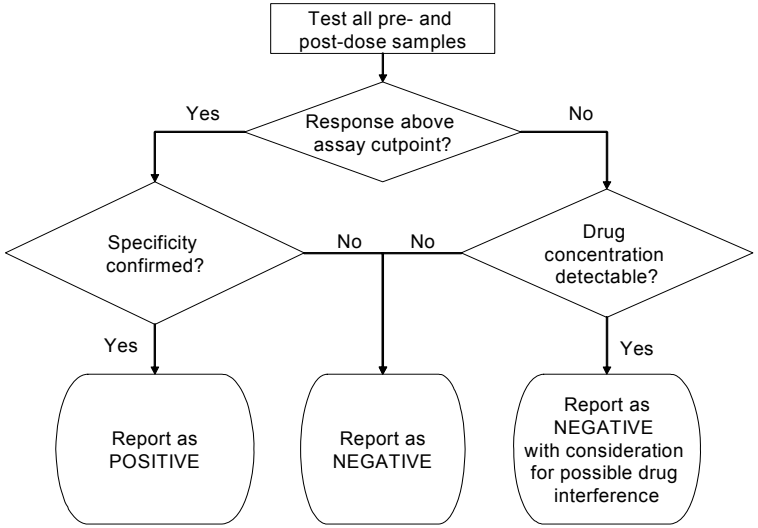

Figure 2. STEP 2: Recommended strategy for characterization of anti-drug antibodies