BIOTECHNOLOGY
INDUSTRY ORGANIZATION
1201 Maryland Avenue SW, Suite 900, Washington D.C. 20024
202-962-9200, www.bio.org

February 2, 2010

Dockets Management Branch (HFA-305)
Food and Drug Administration
5600 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Docket No. FDA-2009-D-0539: Assay Development for Immunogenicity Testing of Therapeutic Proteins

The Biotechnology Industry Organization (BIO) thanks the Food and Drug Administration (FDA) for the opportunity to submit comments on the draft guidance *Assay Development for Immunogenicity Testing of Therapeutic Proteins.*

BIO represents more than 1,200 biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO members are involved in the research and development of innovative healthcare, agricultural, industrial and environmental biotechnology products, thereby expanding the boundaries of science to benefit humanity by providing better healthcare, enhanced agriculture, and a cleaner and safer environment.

GENERAL COMMENTS

We thank the FDA for taking the initiative to generate a guideline addressing assay development for immunogenicity testing of therapeutic proteins. This is a long-awaited document in an area where no or little regulatory advice is available, and given the size and intrinsic complexity of therapeutic proteins, the fact that unwanted immunogenicity has already posed a significant concern with some protein therapeutics, and the challenges in developing adequately characterized methods to detect anti-drug antibodies (ADA), we believe that this guidance document is very important.

The scope of this guidance document is the development of assays addressing a specific type of immunogenicity: immune responses leading to formation of ADA. To make the reader aware of this fact and to avoid confusion, it would be helpful if the introduction contained a definition of the term “immunogenicity”, as it is used in the context of this document, and mentioned that the guidance only deals with immunogenicity testing related to ADA, but not other mechanisms such

as infusion related reactions, or to the clinical interpretation of ADA data. **Recommendation: please provide more clarity on the exact scope of the document.**

The guidance document could be organized in a more cohesive manner. Given that the FDA mentions tiered strategies for immunogenicity testing, and that the general practice is to conduct: a) screening, b) confirmatory, c) neutralizing antibody (NAB) assays in that order, it would be helpful if the guidance document were to also follow that flow. This would help to make the document more clear and logical. **Recommendation: Create major sections on I) screening assays, II) confirmatory assays, III) neutralizing antibody assays, IV) other relevant assays. Under each major section, have a set of subsections, as needed, to address more specific assay considerations. For each section, it would be helpful to have information about the development of a particular type of method followed by a validation discussion, along with any recommendation about when/how such a method should be deployed in the context of a program.**

The new draft guidance provides helpful guidance with regard to development of screening and neutralizing Ab assays, and also how we should deal with immunogenicity assessments for novel therapeutic protein formats, such as fusion proteins. However, the current version of the document does not provide sufficient guidance on the design and validation of confirmatory assays. **Recommendation: Please provide more guidance regarding the design and validation of (immunodepletion/competitive) confirmatory assays.**

The guidance deals in depth with immunogenicity testing as it should be applied to clinical studies. Another major area of immunogenicity testing is in the preclinical setting. This is mentioned occasionally, but the guidance does not clarify what the expectations of the Agency are in this area. **Recommendation: please provide guidance on immunogenicity testing in the preclinical setting.**

The present guidance has many sections where the content is rather like that of a textbook, providing a list of possibilities, instead of making a clear statement about what the current thinking of the Agency is on a particular topic, and what approaches the Applicant should follow. See for example the whole section from line 300 to 323. **Recommendation: please provide more clarity on Agency expectations.**

Assay acceptance criteria are not defined in the guidance. Given that these will often be method specific, some general guidance would be helpful. **Recommendation: please provide some general guidance around assay acceptance criteria.**

Sample time points for immunogenicity testing are suggested by this guidance. These do take into account the fact that many monoclonal antibody therapeutics do not exhibit target-mediated clearance and so generally have very long terminal half lives, so that interference by residual drug can be a problem. However, in the oncology setting, having patients come back 5 half-lives after last dose may not be ethical or feasible. **Recommendation: This should be explicitly acknowledged.**

A “risk based” approach is commonly used for immunogenicity work. Protein therapeutics are categorized as being of high, mid and low risk. This categorization leads to specific choices on

how to design the tiered approach, when to do sample analysis (real time vs. in batches) and so on. Clear differences exist on the need for a NAB assay for an analog of a circulating protein like interferon or erythropoietin and a low-risk human(ized) monoclonal antibody, and greater clarity on risk based assessments and how to operationalize these would be very helpful. For example, the development of a NAB assay may be very technically challenging and resource intensive. Guidance would be appreciated on how to "gate" the timing of NAB assay work. **Recommendation: please provide more Agency input on risk gating of immunogenicity work.**

This draft guidance uses the term Anti-Drug Antibody (ADA), which is quite widely used across the industry and in recent American Association of Pharmaceutical Scientists (AAPS) White Papers on Immunogenicity. However, we, and other companies that are developing Antibody Drug Conjugate (ADC) based therapeutics are now using the term Anti-Therapeutic Antibody (ATA) instead. The word "Drug" has a very specific meaning in the context of an ADC. Here, an Anti-Drug Antibody would be an antibody that is directed against the small molecule/cytotoxic drug component of the ADC, whereas an Anti-Therapeutic Antibody (ATA) would be an antibody that has been elicited by the entire ADC complex. We believe that the use of the term ATA helps eliminate any ambiguity and would like to suggest that this Guidance also adopts this particular terminology. **Recommendation: please use the term Anti-Therapeutic Antibody (ATA).**

Addition of a glossary to the guidance would be useful since several terms are defined in various sections in the main body of the document. This could provide very helpful definitions of terms as they are used in this guidance document: titer, avidity, affinity, rheumatic and rheumatoid factor, replicate (sample replication? Assay replicate?). **Recommendation: please add a glossary to the document.**

We would like to avoid a situation where FDA and the European Medicines Agency (EMEA) provide divergent guidance that will make immunogenicity testing a task even more complex than it is because of its nature (compare with the existing EMEA Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins). We strongly support harmonization of the regulatory effort between the two agencies and with existing AAPS "White Papers." **Recommendation: please consider a "harmonized" global approach to Guidances on immunogenicity testing.**

In general, this guidance does not provide information on how to best assess clinical relevance of ADA data. It merely suggests that ADA incidence and titer data are of clinical utility. Therefore the current guidance is lacking in bridging the key concepts of quantitative and qualitative results that provide clinically relevant information for clinicians on how to interpret immunogenicity data with respect to benefit and risk. We believe that ADA incidence/titer data need to be assessed in the context of data on patient outcomes/safety/efficacy to clarify the impact of immunogenicity in any particular context. The guidance does not identify the adverse events (AEs) that may be considered immunogenicity-related, and about which the agency wants information. The clinical manifestations of antibody- mediated events can be quite varied and can range from transient hypersensitivity responses to fatality. The guidance also does not identify what types of ADA-related statistical analyses are expected at the end of the trial.

Recommendation: These topics are beyond the scope of the current guidance but a future Guidance document concerning how to assess clinical relevance of ADA data could have utility.

Conclusion

Thank you for this opportunity to comment on the draft guidance *Assay Development for Immunogenicity Testing of Therapeutic Proteins***.** BIO provided specific comments on sections of the draft guidance in Appendix 1. In the left column of the table, we identify the line number in the draft guidance; the middle column contains BIO's comments and rationale to support our position; and the right column carries our suggested changes, where applicable (single strikeout for deleted text and underlined type for added text). We would be pleased to provide further input or clarification of our comments, as needed.

Sincerely,

/s/

Katie McCarthy
Director, Science and Regulatory Affairs
Biotechnology Industry Organization (BIO)

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 17 (first occurrence) and throughout the text	The words 'immune assay(s)', 'immune studies' and 'immunoassay(s)' are used to describe the same types of test.	We suggest replacing these words/phrases with the word "immunoassay(s)."
Line 19	The term "binding assays" is used for ADA screening assays.	Please replace "binding assays" with "ADA screening assays" or "anti-drug antibody screening assays."
Line 25	It would be helpful to mention that the guidance does not apply to immunogenicity testing unrelated to anti-drug antibody formation, such as infusion related cytokine release etc.	We suggest the additional language, "…factors that may contribute to immune response rates (immunogenicity) nor immune responses unrelated to formation of anti-drug antibodies."
Line 29	The goal for safety testing should be the same for both types of products. Additionally it should be acknowledged that assays for follow-on biologic therapeutic proteins would be required to meet similar criteria either in this guidance or in future guidances if and when an approval pathway is passed by Congress.	

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 25 and 42	Line 42 refers to an immunogenicity rate.	Please define what is meant by this term.
Line 39-40	The proposed language provides clarity as to the scope of this guidance.	We suggest the additional language, "A variety of effects can arise in patients who develop anti-drug antibodies to therapeutic proteins. These anti-drug antibodies may have no detectable effect; may affect drug disposition and alter exposure to active drug; and/or may elicit a range of adverse health effects, including severe, potentially life-threatening toxicity."
Line 40	The phrase "…at all to extreme harmful effects to patient health" is not grammatically correct.	We suggest the revised language, "… at all to extremely harmful effects ~~to~~ on patient health."
Lines 41-43	Please consider revising this sentence from a simple statement about the need to have immunogenicity rates in the label to something more comprehensive, as rates alone are irrelevant without the corresponding clinical context. For example, a highly immunogenic compound where such immunogenicity does not affect PK/PD, Efficacy, or Safety might be much better than a compound that has relatively low immunogenicity but does have serious safety concerns when present. In such instances, the rates alone would not help physicians to make the necessary benefit/risk decision.	We suggest the revised language, "Because this range exists, clinicians rely on the immunogenicity section of the package ~~labeling~~ insert ~~that contains~~ to provide clinically relevant quantitative and qualitative information regarding a product's immunogenicity ~~rates~~ profile observed during clinical trials."
Line 43-44	Specificity is as important as sensitivity when developing immunogenicity assays.	We suggest the additional language, "This makes the development of valid, specific, sensitive immune assays a key aspect of product development."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 46-50	There is contradiction between what is stated in lines 46-47 (evolving approach) and in lines 49-50 (preliminary validated assays). The statements seem to imply that human assays should be developed at the preclinical stage. However, human assays only have utility for clinical sample analysis.	
Line 49	Factors other than *high risk* can affect whether real-time testing is needed. The need for real-time testing is decided on a case-by-case basis. This line implies that high immunogenicity risk products always require real-time testing.	We suggest the changing "responses are needed" to "responses may be needed."
Lines 48 -49	A definition of "real time data" and when the assay is expected to be run would be beneficial and helpful. We question whether real time patient immunogenicity data are ever really of clinical utility.	Real time sample analysis is only useful if clinical correlations can be proved, and Ad hoc treatment intervention indicated. Please clarify.
Lines 59-61	There is contradiction between the statement here (lines 60-61) and the current practice of the Agency to request information and data at the end of Phase II (EoP2) meeting.	Please provide a unified approach.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 66	Use of the word "parameters" is inappropriate. 'Parameter' refers to boundaries, limits, or ranges, which is not the intended use in this sentence.	We suggest the changing "parameters" to "assay performance characteristics."
Lines 65-72	In head-to-head patient trials, the statement implies that the Applicant should also develop immunogenicity assays for competitor compounds that are of equivalent sensitivity and specificity to the Applicant's assays for its own therapeutic protein. While this might be a possible approach, this is a time- and labor-intensive activity which will not necessarily produce equivalent assays.	We ask for clarification of the statements here.
Lines 69-70	First, it is unclear whether the Agency is referring to follow-on biologic product comparisons, or the invalid comparisons between same-class products. If latter, then the statement "using a standardized assay that has equivalent sensitivity and specificity for both products" is impossible. If an assay can detect ADA to both drugs, it is not specific. Second, how close must the sensitivity values of two assays be to be considered of "equivalent sensitivity?"	Please provide clarity on reference to follow-on biologics versus same-class products. The words "Equivalent sensitivity" should be removed because assay sensitivity is fully dependent upon the positive control used in testing sensitivity. Conclusions on differences in assay sensitivities can be drawn when there are two-three long fold differences, but similarities in sensitivities are going to be elusive.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 75-77	We suggest rewording the sentence, as methodology and sample handling are not assay parameters.	We suggest the following language, "The detection of anti-drug antibodies is dependent on ~~key operating parameters of the~~ assay ~~(e.g.~~ sensitivity, specificity, ~~methodology~~ method used and sample handling~~)~~ procedures which may vary between assays."
Line 76	Use of the word "parameters" is inappropriate. 'Parameter' refers to boundaries, limits, or ranges, which is not the intended use in this sentence.	We suggest the changing "parameters" to "assay performance characteristics."
Lines 79	This is another opportunity to include a focus on the clinical relevance of immunogenicity information. As this section addresses interpretation of immunogenicity results, and especially making comparisons of similar products, please consider adding a statement clarifying the need for perspective on the clinical relevance.	We suggest the following language, "Additionally, any results of immunogenicity testing should be accompanied by a description of the clinically relevant events experienced by patients who had detectable anti-therapeutic antibodies."
Lines 87-88	"…sufficient sensitivity to detect *clinically relevant*…antibodies". The complexity and diversity of the immune response makes immunogenicity rates and titers hard to interpret in the absence of information about "clinically relevant" endpoints.	We request that FDA elaborate on how "clinical relevance" should be established.
Line 91	Interference by the matrix should not merely be evaluated, but avoided.	We suggest the revised language, "Interference……..and this potential effect should be evaluated and minimized, or avoided, if possible."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 91	Soluble drug target can also interfere in assays and cause false-negative or false-positive results, and should be investigated during development/ validation.	We suggest changing "on-board product and this" to "on-board product or target and this."
Line 93	*"Functional or physiological consequences. Immune responses may have multiple effects including neutralizing activity and ability to induce hypersensitivity responses, among others. Immunogenicity tests should be designed to detect such functional consequences."* This implies that assays should be designed which can predict clinical sequelae. However, we do not think that this is feasible.	We suggest replacing the last sentence, "~~Immunogenicity tests should be designed to detect such functional consequences~~" with "Evaluation of functional and physiological consequences of an immune response should be considered a critical part of immunogenicity assessment."
Lines 97-98	A risk-based approach to immunogenicity is of great utility.	We suggest a stronger statement that high-risk products should be treated differently from low-risk compounds. In addition, the guidance should provide more clarity on the risk based approach towards assay development.
Line 100	Currently there is no location within the electronic Common Technical Document (eCTD) structure allocated for immunogenicity, therefore it is unclear as to how an immunogenicity testing paradigm should be provided.	We agree the applicant should provide a rationale for the immunogenicity testing paradigm. It would be helpful if the Agency would provide guidance as to how this should be provided, *i.e.*, whether there is a specific location in the eCTD where this should be provided?
Line 104	Reference # 12 is critical for guidance on immunoassay validations and merits citation here.	We suggest the additional language, "(see section VIII, 1, 2, 12)."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 114	It is unnecessary and irrelevant to stress that "a rapid" screening assay should initially be used.	We suggest deleting "a rapid" or clarify why the screening assay must be rapid.
Line 118	In confirmatory assays IgM antibodies have often been classified falsely as negative due to their low affinity. This would result in underreporting true positive antibodies.	FDA should note that IgM antibodies often appear negative in competition assays due to their low affinity.
Lines 120-125	The approach is presented as a general requirement, which is not the case for low-risk compounds or compounds with extremely low immunogenicity rate.	Please indicate in the statement that the Agency refers to high-risk compounds.
Line 123	"*Further, tests to assess the isotype of the antibodies and their epitope specificity may also be recommended once samples containing antibodies are identified by the screening assay*."	We are not aware that data from assays that determine the isotype of an ATA response ever impact clinical decision making. For this reason we question the utility of characterizing immune responses to therapeutics by isotyping, unless there are extenuating circumstances.
Lines 123-124	Regarding the recommendation to assess epitope specificity, it is unclear that this will add value, particularly if a NAB assay will be run downstream.	Please provide clarity around the utility of epitope specificity testing and under what circumstances it may need to be done.
Line 125	The term *screening* is used here with a different meaning compared to other parts of the guidance.	We suggest using the word "confirmatory" instead.
Lines 127-135	It is implied in the statement that titration is the only acceptable approach.	Please provide clarification of the statement here.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 128	It should be mentioned that it may also be important to know whether an ADA response is transient or persistent.	We suggest the additional language, "…levels is informative. In addition, it may also be important to know whether BAB or NAB formation is a transient or persistent phenomenon."
Line 129	The use of "titer" does not allow a comparison of Ab level with the sensitivity of the assay. This is a significant gap and should be acknowledged. When using mass-based detection platforms such as Biacore™, it is appropriate to report Ab levels as "relative mass units" which is directly comparable to assay sensitivity. We agree that when using ELISA methods the use of mass units can be misleading, however, we believe the guidance should reflect that under some circumstances relative mass units is appropriate	Titers are helpful, but please provide a comment that titer values are affected by the residual drug. In addition, we suggest the following language, "…cut point of the assay.) Mass based detection is an appropriate term only in certain circumstances that allow a direct comparison between amount of antibody in a serum sample and the sensitivity of the assay. Use of relative mass units is sometimes appropriate depending on the type of assay (e.g. Biacore)."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 130-132	In certain cases, expressing neutralizing capacity in terms of the volume of sample required to neutralize a constant biological activity of product can be problematic. The antigen-antibody interaction is a dynamic one and is dependent upon the types of antibodies and their affinities present in a patient sample. An extrapolation of neutralizing capacity from a single or few dilutions of sample tested against a fixed concentration of product can be difficult and yield data that are difficult to interpret. Moreover, assays for this type of determination must use cells that show a robust drug dose response with acceptable accuracy and precision at the various standard curve concentrations employed to generate the curve. These factors are important to allow discernment between true neutralization and assay variability in terms of analytical recovery of the drug concentration used in the assay since a loss of recovery equates to neutralization. Line 294 in the draft guidance also notes that ***"****generally bioassays have significant variability and a limited dynamic range for their activity curves,"*	A Yes/No conclusion for a Nab assay is appropriate. We recommend discouraging the use of this approach (amount of drug neutralized per mL of serum) and believe the most appropriate approach remains the titer-based approach.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
	therefore the approach to use amount of drug neutralized per mL of serum should be discouraged. Also, the co-authors of the current draft of the white paper on NAb assay validation have reached a consensus that this approach has not been widely used within industry and has several limitations. The co-authors of this AAPS-initiated effort to compile the white paper represent several biopharmaceutical companies.	
Line 135	As this section, addresses interpretation of immunogenicity results, and especially making comparisons of similar products, please consider adding a statement clarifying the need for perspective on the clinical relevance.	We suggest the additional language to further provide an opportunity to include a focus on the clinical relevance of immunogenicity information, "Additionally, any results of immunogenicity testing should be accompanied by a description of the clinically relevant events experienced by patients who had detectable anti-therapeutic antibodies."
Line 140	The definition of the sensitivity is based on the available positive control. Thus, it is "relative" sensitivity. Two different positive control antibodies can give quite different estimates of (relative) sensitivity for the same screening assay.	Please clarify that sensitivity is relative to the positive control used for the assay.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 140	First, the initial screening should be very sensitive. '*Very sensitive'* is vague term and contradicts with "*to detect clinically relevant levels of ADA*". Lines 490-491 states "*A sensitivity of 250-500 ng/ml have been associated with clinical events*". If we have a very sensitive assay (e.g., sensitivity of less than 50ng/ml) can the cut point criteria be set to avoid all the issues that arise from having the cut point too close to background? (E.g.: A criteria may be set such as CP set at signal to noise ratio of 2.)	Some guidance on how to deal with assays with low variability (low SD and hence CP too close to background) would be useful.
Line 141	As is, this sentence implies that a false-positive rate should occur during study-phase bioanalysis. That cannot be guaranteed because the false-positive rate was taken into account in the cut point calculation during validation.	We suggest changing, "false positive rate is desirable" to "false positive rate <u>built into the validated screening assay cut point</u> is desirable."
Lines **141** **142** **459**	The term "false positive" is misleading due to the absence of a single, well-defined analyte of interest. We suggest using a more accurate description, e.g. "untreated positive", that focuses on treatment status because this is what is intended.	Lines 141, 142, 459: we suggest replacing "false positive" with "<u>untreated positive</u>". Line 141: we suggest replacing "true positives" with "<u>immunogenicity associated with therapy</u>".

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 145	*"Second, the assay should be able to detect all isotypes (particularly immunoglobulin M (IgM) and the different immunoglobulin G (IgG) isotypes)."* This is unclear terminology: Ig-isotype (Ig-class) and IgG-subtype.	Please provide clarity on why "particularly IgM and IgG" should be monitored. We suggest the revised language, "Second, the assay should in principle be able to detect all isotypes (~~particularly immunoglobulin M (IgM)~~ e.g. IgG, IgE, IgM and the different immunoglobulin G (IgG) subtypes)."
Line 149	Rapidly dissociating antibodies are very common in early immune responses to therapeutic proteins and failure to detect these results in under-reporting of true positive antibody samples.	In addition, all assays, and in particular, "bridging assays" should be evaluated for their ability to detect rapidly dissociating antibodies.
Line 151	Positive control antibodies often do not reflect the type of antibodies that a subject develops in response to therapeutic proteins.	Wherever possible, negative controls should be collected from a non-treated patient population that is representative of the treated patient population. Where possible the control population should have the same underlying disease condition, and should represent a similar gender, age and treatment (except for the study drug) profile so that the underlying profile of the sera/plasma samples are representative of the treated population. Control samples should be collected in the same manner (anticoagulant, volume of sample, sample preparation and storage) as study patient samples.
Lines 148-151 Lines 480 – 482	Control antibody characterization (avidity) as the antibody is a surrogate positive, what is the added value if performing this kind of characterization? Using a stable control antibody (QC) is the key to consistent assay performance.	

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 165	The Bethesda assay is not a screening assay; it is an assay to determine neutralizing antibody activity to the drug.	Therefore, we suggest deleting the Bethesda assay.
Line 167 220	It is important to detect any of these isotypes as they may have physiological relevance.	This list should also include "ability to detect various immunoglobulin isotypes" since RIPA assays lack the ability to detect IgMs. Line 220 does state the limitations of using Protein A, a reagent that is frequently used in RIPA.
Lines 168-169	One of the main differences between the different assay formats is the ability to detect low- vs. high-affinity antidrug antibodies, which depends on the number and vigor of washes.	We suggest the additional language, "on assay sensitivity and ability to detect low-affinity antibodies."
Line 171		We suggest the revised language for clarification, "flurochromes….can result in conformational changes in the antigen which can obscure, destroy, modify or expose relevant antibody binding sites on the protein product in question."
Line 181	The example stated (human serum diluted 1:10 in assay buffer) can be misinterpreted as a critical dilution that must be tested when it is only relevant for assays involving minimal residual disease (MRD) of 10.	We suggest changing the content within parentheses to "(prepared in undiluted serum and subsequently diluted to MRD in assay buffer)."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 182-183	A purified positive control is important for establishing the "relative" (to the positive control) sensitivity, but as a routine control reagent, hyperimmune serum which is substantially diluted (often many 1000s fold) into negative serum should have no confounding matrix effects from the originating species. Because source matrix interference is the main rationale for this recommendation, such large dilutions mitigate that concern.	We suggest using a purified anti-therapeutic Abs for sensitivity determination; use of appropriately diluted hyperimmune serum to a level above the cutpoint can be an alternative approach to use of purified ATAs as positive control.
Line 188	Further alternative for positive control: conjugate of animal anti-drug antibody with human immunoglobulin.	We suggest the additional language, "humanized monoclonal or conjugates of animal-derived positive control antibodies with human immunoglobulin."
Lines 188-192	The use of positive controls derived from patients is an unrealistic expectation due to very limited availability, ethical reasons and practical difficulties in purification	Please delete the sentence, ~~"In some instances, the applicant may be able to generate a positive control antibody from patient samples. While such a reagent can be very valuable, such samples are generally not available in early trials. In addition, an applicant may not be able to generate such a reagent for therapeutic proteins with very low immunogenicity rates."~~
Line 195	Use of the word "parameters" is inappropriate. 'Parameter' refers to boundaries, limits, or ranges, which is not the intended use in this sentence.	We suggest changing "parameters" to "performance characteristics."
Line 195	Interference is a critical assay performance characteristic that should also be validated.	We suggest the additional language, "…sensitivity, specificity, reproducibility, and interference."

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Lines 194-195		We suggest adding "drug tolerance" as one of the key validation parameters.
Line 197	*"These dilutions should be representative of high, medium, and low values in the antibody assay."* Recommending three levels of positive controls seems unnecessary for screening assays since a) controlling the assay near the cut point (low positive) is important when making calls on low positive samples, b) hook effects are assessed during validation. The AAPS white paper mentions only a low positive control. Even a high PC is optional. Here the recommendation for medium PC as well. The mid PC does not add any value, since both screening and titration assessment takes place at the lower end of dilution curve (concentration range). LPC controls the assay sensitivity and HPC shows that there is dependence of the response on the AB concentration. What is the function of medium QC? In PK methods QCs control quantification over the whole range. In ADA quasi-quantification is at cut point.	We believe that two well placed controls (e.g. hi and low) should be adequate. In addition, we recommend low positive control; and consider another positive control at a higher spike level. We request that FDA harmonize the recommendations with existing White Paper concepts (Reference #12 in the guidance document).
Lines 197-199		Please include negative control ('QC zero') samples to the list

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Lines 202-207		We recommend this paragraph should be moved before lines 195-200 to conclude the reasoning.
Lines 204-207	Negative controls are better at approximating the population mean, not the cut point per se as that runs the risk of the negative control becoming positive some percentage of the time. But normalization corrects for negative controls that are lower than the population mean anyway. Since the guidance provides a section on cut point normalization (section VI C), this section is contradictory.	We recommend removing or rephrasing to be consistent with section VI C.
Lines 205-207	The term 'far below the value of the cut point' is too general. Due to the fact that the calculation of the cut point incorporates assay variability (95% one sided limit, or 1.645 SD) the cut point will naturally be above the assay background (i.e., average signal of negative control). Whether it is significantly higher or not is relative. It should be acceptable as long as the overall precision of the assay is acceptable.	We request clarification of the statement here.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 207	Sera profiles for select patient populations may differ from profiles generated from healthy volunteers and may be problematic when used to create a pool of sera to be used as an assay negative control.	We suggest the additional language, "If possible, negative controls are derived from non-treated patients from the same patient population (same disease and treatment profile except for study drug), collected and prepared in the same manner as the study samples."
Lines 209-216	A couple of points about the generation of positive controls in non-human primates vs. other species: • Ethical considerations (including 3R principles) suggest not to use primates for positive control generation • We request more information about the generation of positive controls for immunogenicity testing in animals The draft guidance discourages the approach of generating positive control antibodies using full-length mAb that contain a human Fc region. In certain cases, an appropriate positive control antibody may still be generated using the aforementioned immunization approach by absorbing out the anti-Fc antibodies on an Fc-conjugated affinity column and/or using a FAb ^2-affinity column to enrich the antibody preparation for antibodies that bind to the variable regions of the MAb.	We suggest the revised language, "If non-primate animals are immunized with a monoclonal antibody (mAb) containing a human immunoglobulin constant region (Fc) to develop a positive control, the antibody response ~~is likely to be~~ may be directed against the human Fc ~~and not~~ as well as the variable region." Please provide information on generation of positive controls for animal assays. The guidance text on this topic should allow more flexibility in the use of full length monoclonal antibody immunizations for producing positive control antibodies.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 216	It would be beneficial to provide an example of how to generate positive controls that reflect the immune response anticipated in humans.	We suggest including the following language at the end of line 216: "…, e.g. use of Fab or F(ab)2 fragments for immunization of animals may be advisable."
Line 221	"…antibody bridging studies…" is incorrect here.	We suggest replacing with "antibody bridging assays."
Lines 222-224	Bridging assays are described as having sequential steps starting with antigen (i.e., drug) coating of plates. However, for many years now bridging assays have been done in solution phase, obviating the issues the FDA raises in this regard. The text should mention this as an alternative or even preferred method of doing bridging assays.	We suggest the additional language in middle of line 227: "Alternatively, binding reactions can be performed in solution phase to avoid these limitations, capturing the analyte following formation of the bridging complex."
Line 224	When using direct binding assays it is critical to ensure that the therapeutic protein has been immobilized in a way that all epitopes are reasonably available for binding by anti therapeutic protein antibodies, failure to do so could result in under-reporting of positive antibody samples.	It is important to ensure that multiple epitopes are available upon immobilization of the therapeutic protein. When using direct binding assays it is critical to ensure that the therapeutic protein has been immobilized in a way that all epitopes are reasonably available for binding by anti therapeutic protein antibodies, failure to do so could result in under-reporting of positive antibody samples

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 227	Plate coating density seems an unlikely a cause of missing a low affinity antibody; this is frequently a function of wash steps. However, coating density can impact the ability of binding assays to detect high affinity anti-drug antibodies. Sandwich-type assays also have limitations: • Lower sensitivity for ADA of IgG isotype due to interference by endogenous IgG. • Limitation for therapeutic antibody products: the presentation of the full drug is not possible in case of therapeutic antibody products (anti-human IgG detection reagent would not distinguish between human therapeutic and endogenous IgG).	Please discuss potential limitations of alternative assay format. Therefore, case by case decisions on assay format are necessary, taking in consideration assay specific limitations.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 227-229	"In these assays…." This statement expresses a valid concern, or which assay developers need to be aware, but both assessing it and doing something about it can be an extraordinary challenge. A "critical antigenic determinant" is potentially not just the obvious "active epitope" of a protein (*e.g.*, allostery). Also, since all immunogenicity assay formats involve some labeling and/or immobilization of drug, this statement applies to all assay formats. Yet the practicality of actually determining a) what is a "critical Ag determinant," b) whether a label located near or in such a critical determinant "obscures" makes it extremely unlikely such determinations can be made.	The FDA should acknowledge the complex implications of this simple statement, and perhaps alter the wording "demonstrate" to something like "endeavor to determine."
Lines 248-250	While residual free drug is a very common interfering substance, matrix may contain soluble drug target that can produce false positive results. In fact, drug can sometimes increase the amount of soluble target in matrix. This source of interference needs to be acknowledged.	We suggest the additional language, "The most common matrix interfering substances include residual free drug, soluble multivalent drug targets (which can form bridges with drug and produce false positives in bridging assays or false negatives at high concentrations), and pre-existing auto-antibodies."
Line 250	The term 'product related materials' is somewhat vague.	We suggest using a term defined in other guidance documents such as product related substances, product related degradants, or product related variants.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 252 – 254 **Lines 262 – 264** **Line 402**	Components in the sample matrix can interfere with assay results, but using assay buffer may not provide a relevant look of the matrix effect as assay buffer itself may create high signal due to molecule interactions (ab to ag) under specific biochemical environment (e.g. pH, ion strength, salt concentration, etc).	We suggest deleing, "~~The applicant should also examine this issue … of the protein under consideration~~." We also suggest deleting, "~~Comparing results obtained in buffer alone … for sample testing~~."
Lines 254-256	For monoclonal antibodies used in oncology, the expectation of sample collection after complete wash-out of the therapeutic protein are unrealistic, due to the poor health of the patients and the generally long half-lives of the therapeutic proteins. Does the statement imply, to its extreme, that if such late samples cannot be collected, immunogenicity testing should be waived?	We request clarification of the statement.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 254-256	The exact amount of drug tolerated by an assay (*i.e.*, resistant to interference) is elusive because different ADAs (from different patients) can tolerate different amounts of drug. And, different positive control antibodies also can tolerate different amounts of drug. Therefore, what is the true drug tolerance of an assay? It is not possible to definitively determine the level of drug that does not interfere in the assay. Picking sampling time points when drug level has reduced "to a level where it does not interfere with assay results" based on assay drug tolerance can be misleading.	Please clarify based on the comment provided.
Line 256	Generally therapeutic proteins are not thought of as being able to "decay"; this may be confused with degradation. Instead it is proper to indicate that the protein has undergone several half-lives.	We suggest changing "decayed" to "reduced/cleared."
Line 264	1) This step could reduce the impact of circulating drug in the sample which typically results in under-reporting of true positive samples. 2) Different anticoagulants may have different matrix effects in the assay, affecting the assay sensitivity and linearity.	We suggest the following important considerations: 1) The use of acid dissociation pretreatment may be used to disrupt circulating immune complexes which could increase detection of Abs. 2) Consideration should be given to evaluating different anticoagulant sample collection solutions for their effect on the assay matrix and assay results.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 268 – 270	*"Applicant performs confirmation assays at the screening level."* The reference to "levels" is confusing. "Tier" maybe a more common term to discuss the tiers of testing.	We suggest the revised language, "FDA recommends the applicant perform confirmation assays ~~at the screening level~~ on samples that give a positive result in the screening assay."
Line 270	Titrations do not confirm specificity. Non-specific binding can also be titrated. Antibody depletion does not confirm specific binding either; it only proves that the observed reactivity in the assay was due to antibodies. But the antibody could be binding non-specifically.	We suggest deleting "additional titrations" and "~~antibody depletion~~."
Lines 275 – 298	(*1. Selection of Format*) The wording suggests that a cell-based bioassay is the only NAB format FDA would consider. We are not aware of a general agreement in the bioanalytical community about the superiority of cell-based assay formats for NAB assays. In some cases a ligand-binding assay (LBA) may be a better option if, for example, the LBA is reflective of mechanism of action (MOA) or in vivo situation of the therapeutic.	We suggest an additional paragraph on the appropriateness of alternative NAB assay formats under specific or certain situations.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 283	"*For NAb assays the bioassay should be related to product mechanism of action, otherwise the assay will not be informative as to the effect of NAb on clinical results.*" The guidance should provide more clarity on this statement because guidance previously states, ***"****bioactivity assays are often based on the potency assay,"* and these assays frequently employ artificial readouts such as luciferase expression for measuring drug activity. In addition, FDA should elaborate on the purpose for the "mechanism of action."	We suggest the revised language, "The bioassays should ~~be related to product~~ show relevance to the mechanism of action~~, otherwise the assay will not be informative as to the effect of NAB on clinical results~~ targeted by the product wherever possible."
Lines 297-298	"*We will recommend such assays…"*	Please clarify for which type of product these assays are recommended. It would be helpful to mention the need for a risk-based assessment here.
Lines 300-324	The whole paragraph, including the figure, seems superfluous in guidance ('textbook statement').	Please delete this paragraph.
Line 312	The lower, non-linear part of the curve should also be avoided.	We suggested the proposed changes: Insert a "No" on the lower part of the curve, below the linear part labeled with a "Yes" in Figure 1 (activity curve).
Lines 343 – 345	Antibody depletion may not be definitive for confirming Nab, as non-neutralizing binding antibodies can be depleted as well.	Please provide clarity.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 357-360	*Recommendation d) for Nab-Assays*	The procedure described in paragraph d is not suitable for confirmation of neutralizing activity as the addition of excess product will result in signal change independent of the presence of Nab.
Line 368	Differences in concomitant medications may impact the assay.	We suggest the additional language, "...the product, but, if possible from a matched patient population."
Line 368	To whhat does the phrase "other approaches" refer? Does the FDA mean other assay formats, pre-treatment step, or PK/PD readouts?	Please clarify based on the comment provided
Lines 368 – 369 **Lines 466 – 468**	It may not be realistic to have a discussion with the agency at this level of detail. The timing, plus availability of FDA for consults, may make this impossible.	Please modify the statement
Line 380	editorial: "Critical" instead of "clinical"	editorial: "Critical" instead of "clinical"
Line 388		We suggest using the term, "Produce" rather than "Express."
Line 388	RF may be IgG or A or M.	

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 389		We suggest the revised language, "~~An immunoglobulin "tail"~~An Ig Fc domain, such as with…"
Lines 395-424	It is important to determine an effective MRD during assay development, but it doesn't have to be a component of validation. In validation, the determination of sensitivity in minimally diluted samples adequately supports the MRD.	Please clarify development versus validation
Lines 401 – 402	It is not realistic to achieve a signal very close to that in plain buffer because of the matrix background. The assay buffer may yield high signal of non-specific binding due to molecule interactions under certain desirable biochemical environments (e.g, pH, ion strength, salt concentration, etc.).	We suggest the revised language, "the minimum dilution is the dilution~~ that yields at a signal of non-specific binding of assay diluent~~ at which sample matrix has no impact on the assay results and false response signals of the diluted samples can be avoided."
Line 411	We think that the use of a pool instead of 10 individual samples gives a better chance of simulating what will be observed in the study samples.	Please review the statement.
Line 430		We suggest the revised language, "The decision threshold or cut point..."
Line 430	*"…at or above which"* This is only true for ADA screening assays but not for all NAb assays. It depends on the read-out.	

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 435	First, it is important that the cut point be determined using individual patient samples rather than a pool. Second, because most people use pooled normal human serum (NHS) as a negative control, stating "negative control samples" in this sentence may imply that pooled NHS samples can be used.	We suggest inserting "individual" as indicated, "...determined by using individual drug-naive ADA negative samples."
Line 435	Negative control typically refers to pooled normal serum used for within plate standardization. The term *e.g.* means for example, and therefore it leaves the door open to other types of negative controls.	We suggest replacing "negative control samples" with "drug naïve patient samples" or simply "patient samples". If the Agency prefers to continue to use "negative control samples", we suggest the revised language, "negative control samples ~~(e.g.,~~ samples from patients not exposed to product)."
Lines 437-439	Please clarify that sample size refers to number of patients, and that the inter-patient variability is the key source of variability to be estimated (not "assay variability").	We suggest the revised language, "However, assay validation with ~~a sample size of~~ samples from 50-100 ~~is statistically more reliable for determining the~~ subjects is recommended for statistically reliable estimation of population variability ~~of the assay to effectively define~~ and determination of the assay cut point."
Line 438	At what point in development should sample size be increased for determining assay cut point?.	Please clarify when a sponsor should increase the sample size for determining assay cut point, e.g. during Phase III studies.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 444-454	So-called "outlier" values should NOT be excluded, as a rule, from data analyses related to cut point estimation procedures. Instead, these values should be carefully accounted for in data analyses (e.g. applying empirical percentile estimation to handle "outliers", followed by the fitting of parametric distributions to the remainder of the data set). In general, this will provide a much more reliable estimate of the expected "untreated positive rate" that will be associated with the chosen cut point strategy. Of course, erroneous data values associated with assay or sample handling errors should not be included in data analyses. Using immunodepletion approaches, the applicant should identify those samples with pre-existing antibodies and remove them from the analysis. If the outliers are identified statistically, they should be removed anyway. Further characterization of these outliers using immunodepletion seems unnecessary. Therefore we question whether an immunodepletion assay should be applied to decide whether outliers should be kept or eliminated.	We recommend deleting the entire paragraph, or revising extensively to provide guidance on incorporating outliers appropriately into cut point estimation methods.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 450-452	Clarification is needed regarding the statement "it may be necessary to assign positive responses or a cut point based on the difference between individual patient results before and after exposure." Does this mean that two cut points are recommended – one for identifying pre-existing antibodies and one for identifying post-exposure changes?	Please clarify "it may be necessary to assign positive responses or a cut point based on the difference between individual patient results before and after exposure." Also, the use of titer change should be mentioned (such as a two times increase in titer after exposure may indicate treatment-emergent antibodies).
Lines 458-468	There is no clear indication of the preferred statistical approach to cut point determination is made, especially considering that, with bridging assays, the distribution is almost invariably non-normal (different from the examples reported in the literature, see e.g. Shankar et al).	Please provide additional details on the Agency's current thinking on this subject
Line 459	The cut point is *expected* to yield 5% false-positives.	We suggest the revised language, "While this value is theoretically expected to yield a 5 percent..."
Line 461	The screening assay needs to be confirmed, prior to further analysis.	Only confirmed positive samples should be further analyzed, *e.g.*, by Nab-assay.
Lines 466-467	The statement implies that data should be routinely reviewed with the Agency.	Please clarify whether we have correctly understood the implication of the statement, and if yes, please provide more information about the nature of such routine data review as well as the reasons for it.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 476	The document should acknowledge that the sensitivity of the assay depends on the choice of the positive control. Computing "assay sensitivity" may increase confidence in the assay unrealistically.	Please clarify.
Line 478		We suggest that rather being "reported in mass units," assay sensitivity should be reported in "relative mass units."
Line 489	It is important to ensure that sensitivity is reported for neat matrix, after accounting for the sample dilution	We suggest inserting "undiluted" or "neat" in "antibody detectable/ml of _____ matrix."
Lines 490-491	We question the purpose of having a lower limit in the phrase "approximately 250-500 ng/mL." It might imply that assays need not be more sensitive than 250 ng/mL. The 250-500 ng/mL range was taken from a Mire-Sluis et al. paper, but an assay should be made as sensitive as possible, so we suggest using "≤ 500 ng/ml".	We suggest replacing "approximately 250-500 ng/mL" with "≤500 ng/mL."
Lines 490-491	A recommended screening assay sensitivity of 250-500 ng/mL in the absence of any drug is given in a Mire-Sluis et al. paper. However, such a value is somewhat arbitrary because it is based on the quality of the positive control, which may be rather different in terms of composition from the antidrug antibodies that patients may develop.	Please refer to the existing Mire-Sluis et al. paper and provide additional guidance as needed.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 490	While some assay platforms typically result in sensitivities within this range, MSD ECL assays routinely provide sensitivities ranging from 1 – 20 ng/ml and setting the range as 250-500 ng/ml may not be appropriate for all assay platforms.	We suggest changing approximately to "at least."
Lines 490-492	Sensitivity of assays specifically directed against IgE may be included.	We suggest the additional language, "Assays specific for IgEs should be sensitive in the low ng/ml range since IgE antibody present at those concentrations may be clinically meaningful."
Lines 493-534	Most of the section is 'textbook style' and does not belong in a guidance.	
Line 498	Editorial: "Assess" instead of "Asses."	Editorial: "Assess" instead of "Asses."
Lines 499-500	"Monoclonal antibodies are normally based on IgGs." The statement implies that IgGs should be tested for cross-reactivity (as they belong to the same family as monoclonal antibodies).	We question the validity of the statement and request that it be clarified.
Lines 502-504	Please also mention interference from dimeric or multimeric ligands.	We suggested the additional language, "The applicant should clearly demonstrate that the assay method specifically detects anti-drug antibodies and not the monoclonal antibody product itself, non-specific endogenous antibodies, antibody reagents used in the assay, or dimeric/multimeric ligands of the monoclonal antibody product that can form "bridging" complexes with the product."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 511- 513	In clinical assays that are performed in the background of human serum (at MRD), the matrix should provide enough Fc background. In preclinical studies, it may be important to identify ADAs – even if they are against the Fc backbone – because these ADAs might affect the PK/Tox as well.	Please provide clarity.
Lines 530-533	In the competitive-inhibition based approach to specificity confirmation, drug must be used in excess to assure that even high levels of ADA can be inhibited. When drug is in excess, both high and low positive controls will be inhibited equally. Thus the suggestion that high positive control is inhibited less than the low positive control is erroneous.	We suggest deleting, "There should be a relationship between the quantity of antibody and amount of drug…..by a given concentration of product than the low positive control)."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 536-556	Determination of precision (using instrument response) of controls may be of limited value in the validation of semi-quantitative assays ATA assays. Because of the high day-to-day variability of the raw signal in Ligand Binding assays, and also considering that the instrument response is not the final result; it may be of more value to look at reproducibility of result in validation. For discussion purposes here, reproducibility is defined as characterizing the consistency of the sample result and not the consistency of the instrument response. Reproducibility could be characterized by: 1. Evaluating if screen samples demonstrate consistent response upon multiple analysis, i.e. the consistency that samples will be classified as positive or negative upon repeat analysis. This characterization could also be performed by looking at the consistency of response of screen samples when compared to each other (i.e. linear regression of results between two assays).	

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 536-556	Because Titer Values are often the final reported result of positive samples, characterizing the precision of the titer result of a control could also be of benefit.	
Lines 539-540	Although Sponsors strive for sensitive and precise screening assays, the precision of the assay may not demonstrate "subtle" changes in immune response due to changes in product manufacture.	We suggest rephrasing the sentence, "Assay reproducibly is important for confidence in interpretation of data when assessing immunogenicity across time and multiple studies with various manufacturing lots and patient populations."
Lines 538-548	What type of readout is expected to be reported for precision? Possibilities are concentration, categorical, OD values…	Please specify.
Lines 543-545	We question why six replicates should be used for intra-assay precision. Precision should be evaluated using the same number of replicates as would be applied during study-phase bioanalysis.	Please clarify based on the comment provided

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 544	"*Intra-assay precision should be evaluated with a minimum of 6 replicates per plate.*" Why? Intra-assay precision or simply within-plate variation is a QC issue that should be worked out by the assay developers before assay validation. Assay validation should be concerned with inter-assay precision and not intra-assay precision because clinicians want samples that test positive (negative) to retest positive (negative).	The output of an immunogenicity assay is + or -. Precision as used in a PK assay is not directly applicable. The assay should be designed so that if a sample test negative (positive) it retests negative (positive). For this to be the case it is sufficient to show that scores from untreated patient samples from two assays correlate, that is, the samples with high (low) scores in the initial assay have high (low) scores in the re-assay.
Lines 545-546	There is typically no standard curve for the immunogenicity assay, so there should not be an established assay dynamic range.	We suggest the revised language, "Sample should include a negative control~~s~~ and positive ~~samples~~ controls ~~whose testing yields in the low, medium and high levels of the assay dynamic range~~ with appropriate concentration of antibody."
Line 546	When linearity is already established, we question the the need to apply medium positive controls. Low and high should be enough. What really matters is the low control because variability around it is relevant to the cut point. Since ADA assays are qualitative, variability around the high control is not very relevant.	We suggest omitting the requirement for a "Medium" QC or control.
Lines 550-556	No suggestion is made by the Agency on what is an acceptable value for precision.	Please provide acceptance criteria.
Line 550-556	This paragraph is unclear.	We recommend QC should be a separate section and not mixed with precision.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 554	Normally positional effects are not major contributors to imprecision of the assay. However, plate homogeneity should be demonstrated.	Please correct the statement and stress that plate homogeneity should be tested.
Line 555	It is unclear what "*the applicant should evaluate such effects*" means. It is insufficient if an applicant just evaluates effects and states that there weren't significant positional effects. There will always be some; hence a balanced design approach should be applied during precision determination (as well as cut point determination).	We suggest the additional text, "the applicant should incorporate balanced design testing."
Lines 560-570	No suggestion is made by the Agency on what is an acceptable value for robustness (*e.g.*, effect of hemolysis, lipidemia, bilirubinemia etc.)	Please provide acceptance criteria.
Line 562	Why is a change in buffer considered an example of robustness? Changes in buffer type or composition cannot be considered a "small but deliberate change."	Please delete "buffer" from the sentence.
Line 564	We suggest including tests for short and long term stability, and stability at room temperature.	Please rephrase the sentence including these tests (if considered as important for the evaluation of the reliability of the assay).

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 565 – 568	These parameters should be considered in the assay interference evaluation.	We recommend these be addressed in "2. Specificity and Interference" section.
Line 566	The sample matrix should be defined in the method, and also in the lab procedures in clinical study protocols. Then testing of different anticoagulants is not necessary.	Please provide some guidance on the use of serum versus plasma, and selection of anticoagulants, for ADA assays.
Line 567	Regarding concomitant medication: Oncology patients are treated with drug cocktails of small and large molecules. Should all small and large molecule co-medicines be assessed? Most small molecules do not seem to interfere in ADA assays for protein therapeutics.	Please expand on the subject.
Line 570	The guidance should also include verbiage referencing the number and types of freeze thaw cycles to be thorough.	Sample stability at frozen, refrigerated, and ambient room temperatures should be evaluated.
Line 570	These parameters are critical for surface plasmon resonance (SPR) assays and these criteria are important to evaluate during assay validation.	We suggest adding that SPR assays should also include the validation parameters of surface stability upon regeneration (how many regeneration cycles are supported with a given therapeutic protein) and also parameters around baseline stability.
Line 583		We suggest using the following language, "on the reportable range," rather than "on the linear range."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 604	It may be inappropriate to increase the number of replicates for less tight assays like Nab assays. Increasing the replicate count decreases SD and CV. High variability assays might benefit from the application of more replicates in the assay during study-phase bioanalysis (and thus also tested in validation), but not just in validation alone.	Please clarify based on the comment provided
Lines 616 - 629	The section on Validation of Confirmatory assays is very confusing. The approach discussed in Reference 12 for setting the confirmatory cut point is appropriate. The use of positive control samples for setting the Confirmatory Cut point controls has two problems. First, using the positive control sets the cut point to control the false-negative rate of the assay in detecting the surrogate positive control. Hence, the animal derived positive control has no clinical relevance the cut point is irrelevant. Second, the cut point is determined by the amount of positive control used hence it is arbitrary.	
Lines 618-619	Immunodepletion/competition assays are used to confirm not only the neutralizing assay results but also screening assay results.	We suggest the revised language, "<u>Immunodepletion/competition assays are used to confirm positive results from the immunogenicity testing</u>."

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 626-629	The use of a positive control antibody to determine a specificity confirmation cut point is wrong, and should not be recommended. Using positive controls, and excess drug for inhibition in the competitive assay, very high inhibition values will be achieved (as expected). Such high values will lead to false-negatives.	We suggest deleting, "In this regard, examining percent inhibition of QC samples ... can help to identify meaningful values."
Lines 626-629	QC samples always give large signal quenching on addition of specific proteins, since they are spiked with positive controls that are made by immunization with the specific protein.	We suggest the additional language, "Evaluate the inhibition of signal in a naïve population to establish a confirmatory assay cut point. Confirm the selection of the confirmatory cut point through evaluation of low QC samples." We suggest including cautions regarding the use of naïve population includes the incidence of pre-existing cross-reactive antibodies which will be inhibited by drug. The variations in avidity/affinity of QC samples should also be clearly noted as impacting the inhibition of signal.
Line 638	*"…. will depend on"*	Please also include the dosing regimen and residual drug level.
Lines 638 - 643	"*Optimally, …during the trial,"*	We suggest the revised language, "~~Optimally, …during the trial~~ The applicant should obtain samples at appropriate intervals throughout the trial."
Lines 638-649	Early sampling (7-14 days) for IgM responses can be impractical for long half-life drugs that are likely to be in high concentrations at these time points.	

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 654-655	The use of drug tolerance limit (determined using a positive control) in selecting sampling time points is wrong. This can be allowed only when a panel of monoclonal ADAs of low-to-high affinity have been used to validate a level of drug that is non-interfering. In lieu of such comprehensive validation of drug tolerance, a washout period is necessary.	Please clarify based on the comment provided.
Lines 655-661	While any amount of residual drug can potentially interfere with detecting low avidity/low concentration ATAs, it is often impractical, if not unethical, to obtain washout samples from oncology patients.	We suggest acknowledging the practical limitations of obtaining washout samples in some indications.
Line 661	This step can help reduce the influence of circulating drug contained in samples to be tested for the presence of anti-drug antibodies.	We suggest adding a sentence regarding acid treatment of samples to allow disruption and subsequent detection of Abs that are bound in a circulating immune complex.
Line 659	The statement on products with immunosuppressive function needs clarification.	The following amendment may be included, "…if the product in question is itself an immune suppressant which may interfere with BAB/NAB formation during periods of high exposure but may become immunogenic after treatment when exposure levels are low."
Lines 672-674	How was the value of 1% determined? What statistical tests should be used? How is it done in practice?	Please expand on the subject.
Line 683	This paragraph is not clear. Is it referring to the need for plate-specific cut points?	Please clarify.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 685-693	Normalized cut point is also known as "floating cut point." Furthermore, floating cut points are not always necessary. Reference #12 (Shankar et al., AAPS white paper) provides a logical decision tree for the selection of cut points.	We suggest the mention of the term "floating cut point" in this paragraph, since it is now commonly used to refer to the normalization concept described here. We suggest clarifying that specificity confirmation assay cut point be based on evaluating analytical variability of competitive inhibition of several individual drug-naïve subject matrix samples. See Reference #12 (Shankar et al, AAPS white paper).
Lines 688-690	In the chapter, only normal distribution is considered, while other possibilities, much more common in practice, are not mentioned	Please expand on the subject, including an acknowledgement that non-normally distributed data are quite common.
Line 693	What is an "extreme" case?	Please clarify.
Lines 700-704	Titration is time- and resource-intensive. Are other approaches acceptable?	Please clarify.
Line 701	"*Reciprocal of the dilution able to yield a background just at or above the cut point."*	We suggest replacing "background" with "response, assay response, instrument response".

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Line 704	Some assay systems, such as SPR (Biacore™) are mass-based and therefore, the use of relative mass units is an appropriate measure of the amount of anti-drug antibody contained in a serum sample. This also allows the physician to better understand how much antibody a subject has relative to the sensitivity of the assay.	We strongly believe that the following should be added: "An exception when using relative mass units is appropriate is when using an SPR assay or other assay where the detection is directly based on mass. In these cases it is appropriate to report the level of anti-therapeutic protein antibody in "relative mass units" as this allows a direct comparison of antibody levels with assay sensitivity."
Lines 708-715	It is unclear what an applicant should do to distinguish treatment-emergent ADAs versus pre-existing ADAs. A recommendation should be provided here.	Please provide a recommendation that a two-fold or four-fold increase in ADAs can be considered treatment-emergent.
Line 713	In the section on pre-existing antibodies, it should be emphasized that, in addition to anti-IFN antibodies, there are many other autoantibodies to cytokines and growth factors described in healthy individuals. Accordingly, the reference list should be amended (cf. below).	The following amendment may be included, *"*…For example, autoantibodies to IFN and other cytokines or growth factors, such as IL-8, TNF-alpha, VEGF or G-CSF, can be found…"
Lines 735-748	This is 'textbook style' and does not belong in guidance.	We recommend deleting lines 735-748.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 752-754	The statement on potential neoantigen formation related to fusion proteins is incomplete: It should be made clear that the junction of the components may carry novel T cell epitopes whereas novel B cell epitopes may be formed throughout the whole sequence of the fusion protein as a consequence of the fusion which may generate novel three-dimensional structures composed of elements of both protein components.	We suggest the additional language, "…to measure immune responses to both domains of the parent molecules as well as to the neoantigen, e.g. novel T cell epitopes formed at the junction of the components and/ or novel B cell epitopes formed three-dimensionally by close proximity of domains of both parent molecules."
Lines 762-763	Other methods for assessing immunogenicity, such as ELISPOT, are experimental and it's not clear how best to develop or validate or interpret data from such methods.	Guidance would be appreciated, and needed.
Line 771-772	Measuring IgE in order to predict allergic responses has yet to be shown to have clinical utility for therapeutic product since the patient exhibits allergic responses prior to measurable levels of IgE being present in serum due to their ability to induce an allergic response at very low levels, and with a very short half life.	Please delete the discussion of the need to develop a predictive IgE assay.

line No, paragraph No. or section	Comment and Rationale	Proposed change (if applicable)
Lines 773-778	In this paragraph, anti-drug antibodies of the IgG4 subclass are described as being "less pathogenic" than other Ig isotypes or IgG subclasses. However, we are not sure whether this generalization holds true for most biologics, in light of the fact that several PRCA patients were found to be positive for anti-EPREX antibodies of the IgG4 class after prolonged treatment with the EPREX formulation of Epo.	Please delete or modify the paragraph on IgG4 to reflect this.
Line 783		Please replace "should" with "may."
Line 784	We do not think epitope mapping is always warranted or informative.	Please see comment for line 124.
Line 828	The reference list should be amended.	Please include, "Watanabe M, Uchida K, Nakagaki K, Kanazawa H, Trapnell BC, Hoshino Y, Kagamu H, Yoshizawa H, Keicho N, Goto H, Nakata K. Anti-cytokine autoantibodies are ubiquitous in healthy individuals. FEBS Lett. 2007;581:2017-21."
Lines 834-836	The reference is not complete (outdated: the paper was printed).	Please provide the correct journal reference.