EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 May 2012
EMA/420188/2011
Committee for Medicinal Products for Human Use

Overview of comments received on ' Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use.' (EMA/CHMP/BMWP/86289/2010)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	The Medicines Evaluation Board (MEB-NL)
2	The Janssen Pharmaceutical Companies of Johnson & Johnson
3	*bio*LOGICA Consulting SARL
4	European Generic medicines Association (EGA)
5	European Immunogenicity Platform (EIP)
6	European Bioanalysis Forum vzw (EBF)
7	European Biopharmaceutical Enterprises (EBE)
8	Biotechnology Industry Organization (BIO)
9	The American Association of Pharmaceutical Scientists (AAPS), Therapeutic Protein Immunogenicity Focus Group
10	Pfizer, Inc.
11	F. Hoffmann-La Roche AG

Disclaimer: The comments made by BMWP on the comments sent on the draft guideline were made when preparing the final internal draft. They may in part be outdated following internal consultation of that draft and CHMP discussion and adoption.

Many comments were repetitive or even identical. BMWP did in those cases not repeat their comment.

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1. General comments – overview

Stakeholder no. *(See cover page)*	General comment (if any)	Outcome (if applicable)
1	The principles of this guideline do not differ of the general guideline concerning immunogenicity. In contrast with the statement by the EMA, in our opinion the immunogenic properties of monoclonal antibodies are not unique and shared by other therapeutic proteins like complexity, multiple biological activities and prolonged half-life. So the MEB is not convinced that a specific guideline such as this is necessary and the EMA/CHMP should substantiate its need better or cancel this draft.	Comment not accepted. mAbs do have specific, sometimes unique features e.g., assay problems. Although many aspects of the general guideline apply to mAbs, specific issues for mAbs are not addressed in the general guideline.
	It is suggested that this draft GdL could also be added as Annex to the more general GdL: Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins	Comment not accepted. It is not possible to have annexes to a guideline in the current system.
	This guideline does not directly address non-clinical aspects. In line 57-58 it is stated: "This guideline addresses the major quality and clinical aspects ….". This is agreed. However, in several places reference is made to ''Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins''. Yet, in that guideline, a non-clinical paragraph is included. We would like to comment here that there is a need to reconsider the non-clinical paragraph of this 'general guideline'. There may certainly be a need for immunogenicity testing of biotechnology-derived proteins, including monoclonal antibodies, as is stipulated also in the addendum to the ICH S6 guideline. However, in our view there is no need for immunogenicity testing as part of a comparability exercise. We see that it is possible to detect differences in immunogenic potential in animals between	Comment accepted. The Guideline on Similar Biological Medicinal Products Containing Monoclonal Antibodies - Non-clinical and Clinical Issues indicates that immunogenicity assessment in animals is generally not predictive for immunogenicity in humans, but may be needed for interpretation of in vivo studies in animals. Blood samples should be taken and stored for future evaluations if then needed.

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	proteins in this way. However, we do not see the clinical relevance of such findings. Differences in immunogenicity may indeed indicate that subtle differences may exist between the proteins compared, however, they do not indicate which differences lead to the differences in immunogenicity, nor what the clinical relevance of the observed difference is. Immunogenicity results in animals are very difficult to extrapolate to humans. Consequently, immunogenicity assessment always has to be done in humans, no matter if data from animals already exist.	Since the revision of the ICHS6 guideline (CHMP, July 2011), there is a need to reconsider the non-clinical paragraph of the more general Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins (CHMP, December 2007). This will be taken into account in the next revision.
2	We agree with the general approach of the draft guideline, in particular its emphasis on the myriad factors that can affect immunogenicity and the robust immunogenicity testing it requires both prior to and following market authorization. We urge the EMA to develop additional, specific guidance on the unique issues presented by immunogenicity in biosimilar monoclonal antibodies (mAbs). This draft guideline applies to all mAbs (as well as their derivatives and products of which they are components), whether innovative or biosimilar. The regulation of biosimilars raises additional challenges with regard to immunogenicity. In particular, applicants should establish that the reference product and the proposed biosimilar have similar immunogenicity and should elucidate the implications of immunogenicity on the ability to switch between the reference product and a biosimilar, as well as the implications of switching between the reference product and a biosimilar on immunogenicity.	Comment not accepted. There is a draft biosimilar mAbs guideline. Comment accepted.This should be dealt with in the biosimilar mAbs guideline. Comment not accepted. The issue of switching

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		(interchangeability) is not in the remit of the EMA.
3	Although the draft guideline is highly welcomed in principle, the present format and content is not ideal to provide clear messaging on the product-class considerations for monoclonal antibodies. The detailed comments submitted herewith have the intent of highlighting those aspects of immunogenicity assessment that merit, based on the experience gained with currently marketed products, particular attention to enable a balanced judgement about the impact of undesirable immunogenicity on clinical benefit and risk. The overall recommendation is to abbreviate substantially the length of the text, as well as to re-format the guidance document, in order to emphasise the most pertinent information.	Comment not accepted. The meaning of the comment was not well understood. The risk issue is addressed in detail in a large section of the guideline.
4	1. In general, this is a good and informative document/guideline compiling up-to-date knowledge about immunogenicity. However, it would be very helpful and more user-friendly if a summary of the key guidance and recommendation points is included at the beginning or the end of the document. 2. The guideline contains some abbreviations (e.g. CDR, ECL) that need further explanation. Please consider to include a List of Abbreviations in the document. 3. Specific references included within the document will provide background and insight • Reference immunogenecity CHMP Guideline (14327/2006) in specific sections 4. Biosimilar mAbs should not be treated as a separate class of mAbs 5. From an immunogenecity perspective, biosimilars should be treated like a process change of the originator molecule The draft guideline on biosimilar mAbs places a focus on use of *in vitro*	Comment not accepted. Adding a summary of the guideline is not possible as per the current guideline format. Comment accepted. The abbreviations will be defined. Comment not accepted. References are normally not included in the EMA guidelines. Comment accepted. This is consistent with the guideline.

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	non-clinical studies for making the decision as to what extent of what, if any, *in vivo* work should be required; EGA welcomes this approach, including the opportunity for non-clinical immunogenecity assessments if deemed necessary	
5	The need for a separate guideline for the immunogenicity of monoclonal antibodies needs to be challenged. In our opinion all aspects of the immunogenicity of therapeutic proteins as already discussed in the general guideline (EMEA/CHMP/BMWP/14327/2006) are also applicable to monoclonal antibodies. In many section this guideline does not provide real guidance to the applicant as it is more or less a summary of previous experience. More guidance on the expectations of the agency and how these should be put in practise would be highly appreciated. In some sections the guideline contains discussion of advantages/disadvantages of different assay formats. In our opinion this should not be part of a guideline, especially because methods and technologies are quickly evolving.	Comment not accepted. mAbs do have specific, sometimes unique features e.g., assay problems. Although many aspects of the general guideline apply to mAbs, specific issues for mAbs are not addressed in the general guideline. Comment not accepted. The guidelines can be modified when required to take account of technological advances. General principles of methodologies do not change rapidly. The guideline does not make any recommendations on assay platforms.
6	The EBF acknowledges the need of a document for guidance on assessment of unwanted immunogenicity potentially induced by bioproducts. Comments on this document were collected from the EBF member companies with monoclonal antibody (mAb) products under development. Experts on immunogenicity testing of mAb products of these companies gave their comments as requested by EMA. In general the guideline seems to contain discussion of relative merits of different assay platforms which should be left to individual companies especially since methods and technologies evolve quickly. The overall and most important comment of the EBF is that the major	Comment not accepted. The guidelines can be modified when required to take account of technological advances. General principles of methodologies do not change rapidly. The guideline does not make any recommendations on assay platforms.

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	part of this guideline is not restricted to therapeutic mAb's but applicable to all biologics. Since there is so much similarity between the current guideline and the general immunogenicity guideline (guideline 380 (EMEA/CHMP/BMWP/14327/2006)) it is of high importance that these will not contradict, now as well as in future updates. We therefore strongly suggest filtering the specific mAb issues out of the current guidance and merging them into the existing general immunogenicity guideline. The EBF therefore strongly suggests to combine this draft guidance on immunogenicity testing of mAb products with the already existing guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins. The EBF thanks EMA for drafting this guideline for unwanted immunogenicity testing specified to mAb products and asks the EMA to consider all comments given.	Comment not accepted.There is no contradiction between this guideline and the general immunogenicity guideline. Combining the two guidelines is not considered advisable as it will produce a confusing hybrid of mixed specific and general guidance.
7	EBE welcome the opportunity to comment on the guideline on immunogenicity assessment of mAbs, and support a risk based approach. However, there is no clear consensus within industry about the relevance of the proposed guideline. Consequently, EBE recommend that the final guideline focuses on highlighting the particularities in immunogenicity assessment of monoclonal antibodies. Large parts of the draft guideline are similar to the existing EMA general guidance on immunogenicity. It would be critical to clearly define the scope and the terms "immunogenicity" and "unwanted immunogenicity" in the guideline. Literature references for the claims should be added to the guideline. EBE recommend that the current section 5, on prediction and reduction, should be removed from the final guideline. So far no	Comment not accepted. There is no contradiction between this guideline and the general immunogenicity guideline. Combining the two guidelines is not considered advisable as it will produce a confusing hybrid of mixed specific and general guidance. Comment accepted. These terms are used interchangeably and existing references to immunogenicity will be changed to"unwanted immunogenicity" . Comment not accepted. References are normally not included in the EMA guidelines.

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	method is available to predict immunogenicity in humans. It is mentioned in the introduction that the guideline addresses issues on immunogenicity of biosimilar antibodies. However no guidance on biosimilars is provided. EBE ask for clear and transparent guidance on how immunogenicity of biosimilar mAbs is to be evaluated. Specific issues that could be addressed include the need to understand the implications of potential switching between products and the need to characterize cross-reactivity of antibodies and differences between the products. Besides the unwanted immunogenicity, there may be neutral or even positive effects of immunogenicity. Furthermore, the extent of development of unwanted immunogenicity is highly antibody specific and needs to be weighed against the clinical benefit. See following literature citations: Most important is the therapeutic benefit of the antibody in context with the regimen of administration – "formation of anti-drug antibodies is acceptable if clinical objectives have been met" [1] Development of anti-drug-antibodies is not necessarily associated with an increased safety risk – "20 % of murine-derived antibodies induce negligible/tolerable levels of HAMA" [1] The clinical relevance of immunogenicity is far reaching and highly antibody dependent [1] For some therapeutic antibodies, the development of anti-antibodies is associated with an improved therapeutic effect [2], [3]- [1] Getts, DR et al, Have we overestimated the benefit of	Comment accepted. Comment accepted.This will be dealt with in the biosimilar mAbs guideline. Comment not accepted. The issue of switching (interchangeability) is not in the remit of the.EMA. Comment not accepted.T his point is not considered as general.

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	human(ized) antibodies?, mABs 2:6, 682-694, Nov/Dec 2010 [2] DeNardo GL et al., Characterization of human IgG antimouse antibody in patients with B-cell malignancies. Clin Cancer Res 2003;9:4013–21S. [3] Miotti S et al., Level of anti-mouse-antibody response induced by bi-specific monoclonal antibody OC/TR in ovarian-carcinoma patients is associated with longer survival. Int J Cancer 1999;84:62–8. The executive summary and introduction of the guideline could be revised accordingly.	
9	It is not clear why there is a need for a separate guidance on immunogenicity of mAbs. Much of the content of the draft guidance is not specific to mAbs. There needs to be more clarity on what specific actions should/need not be taken for a mAb therapeutic. Amendment of the existing general guideline with specific guidance that pertains to mAbs may be more appropriate.	Comment not accepted. mAbs do have specific, sometimes unique features e.g., assay problems. Although many aspects of the general guideline apply to mAbs, specific issues for mAbs are not addressed in the general guideline. More specific guidance on mAbs was also requested before by several stakeholders.
8	The Biotechnology Industry Organization (BIO) thanks the European Medicines Agency (EMA) for the opportunity to submit comments on the draft "Guideline on Immunogenicity Assessment of Monoclonal Antibodies Intended for In Vivo Clinical Use." BIO represents more than 1,100 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	Comments noted.

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	humanity by providing better healthcare, enhanced agriculture, and a cleaner and safer environment. In Section 2 below, BIO provides specific comments on sections of the draft guidance. In the left column of the table, we identify the line number in the draft Guideline; the middle column contains BIO's comments and rationale to support our position, and carries our suggested changes, where applicable (single strikeout for deleted text, and bold, underlined type for added text). We would be pleased to provide further input or clarification of our comments, as needed	
10	It is not clear why there is a need for a separate guidance on the immunogenicity of mAbs. Reason: Much of the content of this draft guidance is not specific to mAbs. As such, it may be more appropriate to provide an addendum to the existing guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins, to provide specific guidance for mAbs.	Comment not accepted. mAbs do have specific, sometimes unique features e.g., assay problems. Although many aspects of the general guideline apply to mAbs, specific issues for mAbs are not addressed in the general guideline.
11	Publication of a guideline on the specific considerations on immunogenicity assessment of monoclonal antibodies (mAbs) is appreciated. Please see below a few general comments on the draft guideline for your consideration.	Comment accepted
11	In this draft guideline, general principles regarding immunogenicity of therapeutic proteins are discussed that should rather be included in the EMA *Guideline on immunogenicity assessment of biotechnology derived therapeutic proteins* (EMEA/CHMP/BMWP/14327/2006) that applies to all protein products. We suggest focusing in the new guideline on issues of immunogenicity that are specific to monoclonal antibodies, and potentially to update the existing general	Comment not accepted. Some general issues apply to all products and are necessary for ensuring consistency with the general guideline

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	immunogenicity guideline to provide adequate guidance on all products. The guideline would also benefit from a more clearly defined scope, i.e. unwanted immunogenicity of mAbs.	All aspects of the new guideline apply to mAbs.
11	The key terms "immunogenicity" and "unwanted immunogenicity" should be defined and explicitly explained in the beginning of the guideline to avoid misunderstandings. In this guideline, "Immunogenicity" is related to humoral immune responses only, i.e. to the formation of anti-therapeutic antibodies (ATAs).	Comment accepted. These terms are used interchangeably and current references to "immunogenicity" will be changed to "unwanted immunogenicity"
11	In our opinion the draft guideline does not provide sufficient guidance on novel mAb formats. Different types of mAb derivatives (for example, F(ab) fragments or antibody drug conjugates) may differ from mAbs in terms of potential mechanisms of immunogenicity and/or the potential impact of immunogenicity on efficacy and safety, and some guidance on how to address this would be very helpful in the guideline.	Comment not accepted. This issue is too specific to be addressed in the guideline.
11	It would be important for this guideline to provide guidance on how immunogenicity information on biosimilar mAbs (and biosimilars to mAb derivatives) should be generated, and also on how information from originator mAb products may be leveraged, particularly in terms of risk assessment.	Comment not accepted. This will be dealt with in the biosimilar mAbs guideline. It is also rather unclear why this would be needed in this guideline, as this issue is dealt with in the overarching guideline and other guidelines e.g. the one on non-clinical and clinical issues.
11	The guideline, in conjunction with other relevant guidelines, defines the requirements for mAb therapeutic immunogenicity assessment at "the final development stage (MAA stage)" of mAb development. However, key decisions often need to be made around immunogenicity at a much earlier stage in mAb development. Although some of the principles described in the guidance can be applied to the earlier stages of drug development, regulatory requirements on immunogenicity assessments at these earlier stages are not well	Comment not accepted. The meaning of "earlier stages" is not well understood. The guideline clearly reflects the requirements for getting a marketing authorisation but some parts of the guideline apply to earlier stages in the product development. This is mentioned in the "scope" section of the guideline.

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	defined. Some elaboration on early stage work would be helpful due to the lack of information for adequate risk assessment (see specific comments on lines 305-306).	
11	Clarification is asked about the EMA's view on the general "risk" of therapeutic monoclonal antibodies. Potential approach of assuming all mAbs have "high" immunogenicity risk is not consistent with the current and widely used industry practice of using a risk based assessment on immunogenicity approach for each therapeutic protein (Koren et al, Shankar, Rosenburg & Worobec).	Comment not accepted. This issue is dealt with in the risk section. The industry approach is not necessarily that of the EMA/CHMP.
11	The unpredictable nature of the immune response from one individual to others and the myriad of factors involved in predisposing/eliciting an immunoglobulin response is recognized throughout the document. Unfortunately, the agency also implies that the industry is to have assays in place throughout the clinical development and also post authorization commitments to examine any patient response, regardless of the clinical relevance.	Comment not accepted. The point made with this comment is not well understood..
11	Clarification around the requirements for neutralizing antibody (NAb) assays for therapeutics is necessary. There is an assumption throughout the guidance that, when a neutralizing antibody response is detected in a NAb assay, there is always a concomitant loss of efficacy. However detection of neutralizing antibodies in an *in vitro* assay does not necessarily mean that there will be a neutralizing antibody response *in vivo*, as the stochiometry of these 2 situations are quite different. In addition, clarification around when not to have NAb assays would be very helpful.	Comment not accepted. This assumption is not stated in the guideline. Comment not accepted. NAb assays are not necessary when no binding antibodies are detected.
11	We ask to consider including appropriate references to scientific	Comment not accepted. References are normally not included

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	publications for the statements and claims in the document to improve understanding of the given guidance and immunogenicity in general. In addition, we ask removing of controversial and not yet proven scientific aspects from the guideline (please see e.g. comments on section 5 below).	in the EMA guidelines. Comment not accepted. It was not considered appropriate to delete this.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
35-41	3	Comment: The justification for this guideline should be that monoclonal antibodies as a product class represent a different balance of risk factors from other classes of therapeutic proteins. Accordingly, the "Executive Summary" should acknowledge this, in explaining that the elements of the immunogenicity risk assessment process may need to be weighted differently to reflect the specific structural and functional properties of monoclonal antibodies. Proposed change (if any): Replace existing text with: "*This guideline seeks to illustrate how the specific structural and functional properties of monoclonal antibodies should influence the assessment of immunogenicity-related risks, taking into account the general considerations described in the "Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins" (EMEA/CHMP/BMWP/14327/2006, adopted April 2008, referred to henceforth as 'the general guideline')."*	Comment not accepted. Comment not accepted. This is a biased view which does not take account of all factors that need to be considered while assessing unwanted immunogenicity. The guideline has a significant risk section which discusses this.
36	1	Comment: As long as we do not understand the mechanisms of the immunogenicity of therapeutic proteins, including	

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		monoclonal antibodies, the adjective "unwanted" is premature and too suggestive that regulatory agency want companies to do everything they can do to avoid immunogenicity. Immunogenicity could be part of a more general regulatory mechanism and the best we could achieve (and regulators could ask for) is to reduce the clinical risks associated with immunogenicity. Proposed change (if any): Leave out "unwanted" here and in the rest of the guideline	Comment not accepted. The immunogenicity is "unwanted" and most comments prefer the use of this term.
42-58	3	Comment: The purpose of the introductory section should be to preface the issues to be addressed in a guideline dedicated to the immunogenicity risk assessment of monoclonal antibody products, namely: • What particular risk factors should be considered as being more or less relevant for monoclonal antibodies compared with other therapeutic proteins? • Are there special considerations for detection and evaluation of the probability of host anti-drug antibody responses? • What are the most likely clinical consequences, and how might clinical impact best be assessed in the pre- and	Comment partially accepted. This part of the comment is not accepted. This is a biased view and does not take account of all factors that need to be considered while assessing unwanted immunogenicity. The guideline has a significant risk section which discusses this. This part of the comment is accepted. All mentioned aspects have been considered in the guideline.

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		post-marketing phases? Proposed change (if any): Replace existing text with: "*It is recognised that monoclonal antibodies induce undesirable immunogenicity, such that the principles described in the general guideline are applicable. Accordingly, the Applicant will need to provide a balanced assessment of the level of risk posed by undesirable immunogenicity, based on an identification of risk factors and evaluation of the rate of occurrence and severity of the clinical consequences.* *The experience gained for currently marketed monoclonal antibody products indicates that the following risk factors merit particular consideration to enable a balanced assessment of the clinical impact of immunogenicity-related risks of this product class:* • *Primary amino acid sequence* • *Post-translational modification* • *Product or process-related impurities* • *Nature of target antigen* • *Capacity for direct activation of innate immune system* • *Conditions of clinical use* *The relatively high level of molecular complexity of*	

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		monoclonal antibodies can create uncertainty regarding the technical evaluation of host anti-drug antibody responses. This guideline provides advice about the nature of the bioanalytical methods that are best suited to monitor immunogenicity in non-clinical and clinical studies. *Finally, guidance is provided on the clinical parameters that should be correlated with bioanalytical signals in order to assess the clinical impact of undesirable immunogenicity of monoclonal antibodies."*	
47-49	1	Comment: No arguments are given why this specific guideline is necessary	Comment not accepted. mAbs do have specific, sometimes unique features e.g., assay problems. Although many aspects of the general guideline apply to mAbs, specific issues for mAbs are not addressed in the general guideline.
47-49	4	Comment: The wording of this sentence starting with "However, some specific aspects…" is suggestive that biosimilar mAbs are a separate class of mAbs having other specific aspects regarding immunogenicity than non-biosimilar mAbs., Firstly, this suggestion has no scientific basis and secondly, biosimilars are not mentioned in the rest of the guideline at all. Therefore, the reference to biosimilars should be deleted. Proposed change (if any): Delete as follows: "*However, some specific aspects of immunogenicity are*	Comment not accepted. It is not suggested that biosimilars are different – it is just stated that they are also within this product class.

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		exclusively or primarily relevant for mAbs or novel mAb derivatives (e.g. Fab fragments, scfv, nanobodies, minibodies) ~~or biosimlar mAbs~~ and these are addressed in this guideline."	
47-49	7	Comment: This text implies that the guidance will address not only mAbs, but also mAb derivatives (Fab fragments, ADCs etc). However there is very limited information about such mAb derivatives later in the guidance. Proposed change (if any): Please provide more guidance on this topic.	Comment not accepted. This level of details is not appropriate for a regulatory guideline.
47-49	11	Comment: This text implies that the guidance will address not only mAbs, but also mAb derivatives (Fab fragments, ADCs etc). However there is very limited information about such mAb derivatives later in the guidance. Proposed change (if any): Please provide more guidance on this topic.	Comment not accepted. This level of details is not appropriate for a regulatory guideline.
49	5	Comment: The wording of this sentence starting with "However, some specific aspects…" indicates that biosimilar mAbs are a separate class of mAbs having other specific aspects regarding immunogenicity than non-biosimilar mAbs. However, this suggestion has no scientific basis and therefore the wording should be changed.	Comment not accepted. It is not suggested that biosimilars are different – it is just stated that they are also within this product class.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Delete "biosimilar mAbs" as follows: "However, some specific aspects of immunogenicity are exclusively or primarily relevant for mAbs or novel mAb derivatives (eg Fab fragments, scfv, nanobodies, minibodies) ~~or biosimlar mAbs~~ and these are addressed in this guideline."	
51	7	Proposed change (if any): Modify sentence: ~~Many~~ mAb products are known to be associated with ~~unwanted~~ immunogenicity <u>which is</u> ~~and~~ in some cases unwanted ~~the immunogenicity~~ <u>as it</u> causes impaired clinical responses or rarely serious adverse reactions which require clinical intervention.	Comment not accepted.This comment is considered incorrect.
52-53	4	Comment: To sentence "… and in some cases the immunogenicity causes impaired clinical responses…" Immunogenicity may lead to impaired response is in our opinion a more correct way to express, as one can experience gradual or sudden lowering or complete loss of response. Proposed change (if any): "*…in some cases the immunogenicity ~~causes~~* ***may lead to*** *impaired clinical responses …"*	Comment not accepted.The proposed modified text does not differ from the original text.
53	1	Comment: Infusion reactions to monoclonal antibodies are	

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		relatively common and have a high association with induced antibodies Proposed change (if any): Delete "rarely"	Comment not accepted. The guideline specifies serious adverse reactions which are somewhat rare.
53	5	Comment: Infusion reactions to monoclonal antibodies are relatively common and have a high association with induced antibodies Proposed change (if any): delete "rarely"	Comment not accepted. The guideline specifies serious adverse reactions which are somewhat rare.
54	7	Comment: Therapeutic area indications should be considered when addressing immunogenicity.	Comment not accepted. This level of details is not appropriate for a regulatory guideline.
54	11	Comment: Therapeutic area indications should be considered when addressing immunogenicity. Proposed change (if any): Guidance should be provided in this document for distinct classes of mAbs such as agonists or antagonists, products for oncology (immune suppressed patients) vs. products for chronic dosing in non-immune suppressed populations etc.	Comment not accepted. This level of details is not appropriate for a regulatory guideline.
55-58	8	**Comment:** The statement, "This guideline addresses the major quality and clinical aspects that are important to consider in order to adequately address the problems	Comment accepted.

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		with detection of and risk related to the development of an immune response to the particular mAb in the particular clinical indication sought," is a very good description of the proposed scope of the document. **Proposed change:** We suggest moving this sentence into the "Scope" (Line 60).	
55-58	7	Comment: The statement "This guideline addresses the major quality and clinical aspects that are important to consider in order to adequately address the problems with detection of and risk related to the development of an immune response to the particular mAb in the particular clinical indication sought" is a very good description of the proposed scope of the document. Proposed change (if any): We suggest moving this sentence into the "Scope" (Line 60).	Comment accepted.
57	4	Comment: Some immune responses of mAbs may be desired. Therefore, please specify here that unwanted immune responses are meant. Proposed change (if any): Insert "unwanted" as follows: *"…and risk related to the development of an **unwanted** immune response to the particular mAb in the*	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		particular…"	
61	7	Comment: The term "immune response": care should be taken that different immunological concepts are not mixed; "immune responses" should be replaced by " development of ATA", i.e. the formation of antibodies	Comment not accepted. Immune responses are induced, antibodies are measured to assess this.
61	11	Comment: The term "immune response": care should be taken that different immunological concepts are not mixed up; "immune responses" should be replaced by "immunogenicity", i.e. the formation of antibodies	Comment not accepted. Immune responses are induced, antibodies are measured to assess this.
62-63	4	Comment: The guideline is specified for mAbs. Please consider to extend it also to complex fusion proteins in general (-cept molecules). Proposed change (if any): *The guidelines applies to mAbs, their derivatives, ~~and~~ products of which they are components, e.g. conjugates* ***and related substances like for example fusion proteins or IgG Fc (-cept molecules)."***	Comment accepted.
62-63	5	Comment: The guideline is specified for mAbs. Please consider to extend it also to complex fusion proteins in general (-cept molecules). Proposed change (if any):	Comment accepted.

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		Please add the following sentence: "While this guidance is specifically related to mAbs, the principles discussed may also, on a case-by-case basis, be relevant for related substances like for example fusion proteins based on IgG Fc (-cept molecules)."	
66-73	1	Comment: Here a number of guidelines are mentioned. Yet regarding immunogenicity assessment of proteins, including mAbs, in animals the most relevant reference document should be the Addendum to the ICH S6 guideline on the non-clinical evaluation of biotechnology-derived pharmaceuticals. Proposed change (if any): Include a reference to the ICH S6 addendum	Comment accepted.
72	5	Comment: Change wording please see below: Proposed change (if any): This guideline is primarily aimed at products in clinical development (up to marketing authorization…)"	Comment partly accepted. This is already considered in guideline.
72-73	5	Comment: Please be more specific on the principles which are more relevant to the earlier phases	Comment not accepted. This level of details is not appropriate for a regulatory guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
72-73	11	Comment: These lines indicate that the guidance will focus on the final development stage (MAA stage) of mAb development. However, key decisions need to be made around immunogenicity at a much early stage in mAb development, and guidance on the earlier phases of drug development would also be helpful. Please outline why no recommendations on the pre-clinical phase are provided. Proposed change (if any): Please provide guidance on earlier development phases also.	Comment not accepted. The Guideline on Similar Biological Medicinal Products Containing Monoclonal Antibodies - Non-clinical and Clinical Issues indicates that immunogenicity assessment in animals is generally not predictive for immunogenicity in humans, but may be needed for interpretation of in vivo studies in animals.
78-79	8	**Comment:** The title of this section is misleading. It does not explain or describe variability of immunogenicity as much as it instructs on the considerations for the potential causes of development of unwanted anti-drug antibodies. **Proposed change:** We suggest the following title: "**Considerations for development of unwanted immune responses,**" or "**Factors affecting the monoclonal antibody immunogenicity.**"	Comment accepted.
78-124	3	Comment: This section does not provide any guidance for the Applicant. On the contrary, it presents some general statements about risk factors in a rather unstructured manner that may be confusing for the reader.	Comment partly accepted. These sections had indeed too much overlap with the general guideline on immunogenicity of therapeutic proteins. The entire clinical/risk part has been rewritten.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): This section could be deleted. The subsequent sections of the guideline could then be re-formatted using the following sequence of sub-headings: 4. Identification of risk factors 4.1 Primary amino acid sequence 4.2 Post-translational modification 4.3 Product- or process-related impurities 4.4 Nature of target antigen 4.5 Capacity for direct activation of innate immune system 4.6 Conditions of clinical use 5. Bioanalytical evaluation of immunogenicity of mAbs 6. Assessment of clinical impact 7. Risk mitigation	
78-124	5	Comment: The aim of this whole section is not clear. It summarizes text book knowledge on immunogenicity. A huge part of it is also redundant with section 9. Proposed change (if any): Merge content with section 9. If you consider to keep	Comment partly accepted. These sections had indeed too much overlap with the general guideline on immunogenicity of therapeutic proteins. The entire clinical/risk part has been

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		this section please consider the following comments:	rewritten.
78-124	7	Comment: The whole section 4. provides points to consider for therapeutic protein development and immunogenicity development and only very few aspects (e.g. CDR specificity of ATAs) deal with mAbs. Proposed change (if any): Shorten the section and focus on mAb specific considerations	Comment not accepted. The proposal is not clear enough.
78-124	11	Comment: The whole section 4. provides points to consider for therapeutic protein development and immunogenicity development and only very few aspects (e.g. CDR specificity of ADAs) deal with mAbs. Proposed change (if any):Shorten the section and focus on mAb specific considerations	Comment not accepted. The proposal is not clear enough.
82-84	1	Comment: As all therapeutic proteins induce antibodies, an immunogenic response is highly predictable. Only the incidence and the level of the antibody response is difficult to predict. Proposed change: Reformulate sentence	Comment accepted.
82-84	5	Comment:	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		As all therapeutic proteins induce antibodies, an immunogenic response is highly predictable. Only the incidence and the level of the antibody response is difficult to predict. Proposed change (if any): Rephrase sentence	
84	4	Comment: Some immune responses of mAbs may be desired. Therefore, please specify here that unwanted immune responses are meant. Proposed change (if any): *"… to provoke an **unwanted** immunogenic response."*	Comment not accepted. The guideline deals with unwanted immunogenicity.
87-90	1	Comment: What is: "identical to the endogenous human amino acid sequence"? What is " a primary sequence"?	Comment accepted. Sentence will be rewritten/deleted.
87-90	7	Comment: Since mAbs contain CDR regions, it is not possible for mAbs to have heavy and light chain amino acid sequences that are completely identical to those of endogenous human immunoglobulins. Proposed change (if any): Sentence should be reworded, do not use word 'identical".	Comment accepted.
87-90	11	Comment: Since mAbs contain CDR regions, it is not possible for mAbs to have heavy and light chain amino acid sequences that are completely identical to those of	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		endogenous human immunoglobulins. Proposed change (if any): Sentence needs to be reworded.	
90	7	Comment: In some cases, anti-therapeutic antibodies (ATA) may be induced by conformational epitopes as well as (or instead of) ATAs induced by linear epitopes. Proposed change (if any): Please clarify in the text	Comment not accepted.The existing text is consistent with this comment.
90	11	Comment: In some cases, anti-therapeutic antibodies (ATA) may be induced by conformational epitopes as well as (or instead of) ATAs induced by linear epitopes. Proposed change (if any): Please clarify.	Comment not accepted.The existing text is consistent with this comment.
90-92	1	Comment: This sentence about the anti-CDR response is very cryptic. The EMA/CHMP seems to mix up the target with the cause of the immune response. Proposed change (if any): Delete sentence	Comment not accepted.This comment is not well understood.
90-96	8	**Comment:** As mentioned on line 94, emerging constructs and framework variations may challenge the statement in line 91 regarding the immune response	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		being predominantly anti-idiotypic. The statement in line 91 may be taken out of context. Revised wording would also support the statement on line 115, "Furthermore, previous exposure to similar or related monoclonal antibodies can also influence immunogenicity." **Proposed change:** "In such cases, especially with humanised or human sequence mAbs the immune response ~~is predominantly~~ **may be** anti-idiotypic ~~(as the CDRs are unique in sequence for mAbs), which clearly can compromise clinical responses to the mAb." In some cases, antibodies can be induced against the constant region of human or humanised mAbs and this can affect the immunobiological function of the mAb. There is less experience with clinical use of emerging constructs and these may add to the perception of risk,~~ **however, with the increased clinical use of emerging constructs, exclusive specificity to the CDR region cannot be assumed.** Special consideration should be given to next generation products, for example, bivalent mAbs."	The wording was changed in the guideline.
90-96	7	Comment: As mentioned on line 94, emerging constructs and framework variations may challenge the statement in line 91, regarding the immune response being predominantly anti-idiotypic. The statement in line 91 may be taken out of context. Revised wording would also support the statement on line 115 "Furthermore,	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		previous exposure to similar or related monoclonal antibodies can also influence immunogenicity". Proposed change (if any): "In such cases, especially with humanised or human sequence mAbs the immune response ~~is predominantly~~ may be anti-idiotypic ~~(as the CDRs are unique in sequence for mAbs), which clearly can compromise clinical responses to the mAb." In some cases, antibodies can be induced against the constant region of human or humanised mAbs and this can affect the immunobiological function of the mAb. There is less experience with clinical use of emerging constructs and these may add to the perception of risk~~, however, with the increased clinical use of emerging constructs, exclusive specificity to the CDR region cannot be assumed. Special consideration should be given to next generation products, for example, bivalent mAbs."	
92-94	7	Comment: Syntax error and lack of clarity in "There is less experience with clinical use of emerging constructs and these" Proposed change (if any): Please re-word to "There is less clinical experience with emerging mAb based constructs and this may…."	Comment accepted.
92-94	11	Comment: Syntax error and lack of clarity in "There is less experience with clinical use of emerging constructs and	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		these" Proposed change (if any): Please re-word to "There is less clinical experience with emerging mAb based constructs and this may…."	
93-94	4	Comment: It is not clear what is meant with "immunobiological function of the mAb". Whether the anti-mAb antibody is against variable (CDR) or constant regions, it can affect both the function and/or the clearance (PK). Proposed change (if any): *"…and this can **also** affect ~~the immunological function of the mAb~~ **the antibody effector function(s) of the mAb as well as the clinical response including PK and PD.***	Comment accepted.
93-94	5	Comment: It is not clear what is meant with "immunobiological function of the mAb". Does "immunobiological function of the mAb" stand for effector function (i.e. ADCC; complement binding)? Whether the anti-mAB antibody is against variable (CDR) or constant regions, it can affect both the function and/or the clearance (PK). Proposed change (if any): Please modify, e.g. as follows:	Comment accepted. This statement was modified.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		"... and this can also affect the ~~immunobiological function~~ clinical response and the clearance of the mAb." If you will decide to keep the sentence please use "effector function" instead of "immunobiological function".	
96	7	Comment: All conventional mAbs are bivalent. To add clarity and give the correct meaning here, the text should say heterovalent or bi-specific, rather than bivalent. In addition, please specify the "special considerations" for next generation mAb based products. Proposed change (if any): Please change text from "bivalent" to "heterovalent" or "bi-specific". Specify "special considerations".	Comment accepted.
96	11	Comment: All conventional mAbs are bivalent. To add clarity and give the correct meaning here, the text should say heterovalent or bi-specific, rather than bivalent. In addition, please specify the "special considerations" for next generation mAb based products. Proposed change (if any): Please change text from "bivalent" to "heterovalent" or "bi-specific". Specify "special considerations".	Comment accepted.
97	1	Comment: Formulation/Container/Storage issues for immunogenicity are known for non-mAb therapeutics. Please consider whether there has been relevant mAb	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		specific experience and provide specific guidance on mAb therapeutics.	Comment not accepted. There is not enough experience regarding this issue.
97	11	Comment: Formulation/Container/Storage issues for immunogenicity are known for non-mAb therapeutics. Please specify whether there has been relevant mAb specific experience and provide specific guidance on mAb therapeutics.	Comment not accepted. There is not enough experience regarding this issue.
97-107	1	Comment: The problem of this paragraph is that most of the factors are speculation. There are no scientific data supporting that impurities, different glycosylation, deamidation, adjuvants from containers, etc. are real risk factors Proposed change (if any): Separate facts from speculation	Comment partly accepted. This could be considered as speculation, but it is based on the existing experience with non mAb products.
97-107	5	Comment: Most of the factors described in this paragraph are speculation. To our knowledge there are no scientific data supporting that impurities, different glycosylation, deamidation, adjuvants from containers, etc. are real risk factors for immunogenicity Proposed change (if any): We would propose to clearly indicate (e.g. by reference to literature) which factors are proven to promote an	Comment partly accepted. This could be considered as speculation, but it is based on the existing experience with non mAb products. Including references is not appropriate for a regulatory guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		immune response and to discriminate them from the ones that are rather based on speculation	
97-107	10	Comment: Although, several examples are given of potential attributes which may impact the immunogenicity profile of the product, further clarity is needed. For example, 'deamidation' is specifically called out as being potentially immunogenic; however, there is no substantial evidence to point to deamidation as being immunogenic. Furthermore, impurities do not arise from the route, dose or frequency of administration. Rather, impurities should be evaluated relative to the route, dose and frequency of administration (in addition to the patient population and indication) to fully assess the risk of immunogenicity of a specific impurity. Proposed change: Multiple factors can contribute to immunogenicity, including impurities arising from the process and product. Specific examples of factors which may influence the immunogenicity profile of the product include the formulation, container closure system and storage conditions. Additional process and product related impurities can also influence the immunogenicity profile. Specific examples include foreign glycosylation patterns, aggregation and particulates.	Comment accepted. The example of deamidation was deleted. The guideline is consistent with these comments.
97-124	9	Comment: These paragraphs contain information that appears to	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		be pertinent to all protein biotherapeutics and is not unique to mAbs. Proposed change: Delete sections and replace with reference to follow general guideline.	Comment not accepted. These paragraphs are relevant to mAbs.
97-124	10	Comment: These two paragraphs contain information that appears to be pertinent to all protein biotherapeutics and not unique to mAbs. Proposed change: Delete the above paragraphs and replace with reference to the existing general guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins, to provide specific guidance for mAbs.	Comment not accepted. These paragraphs are relevant to mAbs.
100-101	4	Comment: "Deamidation" is mentioned as an example for a modification that might influence immunogenic properties. Actually, we believe that this example is not correct as we are not aware of any case where the immunogenicity of a therapeutic protein has been modified by deamidation. This statement is therefore misleading as it might create a perception which is not founded on data. Proposed change (if any): *"… e.g. modification of protein conformation, extraction of impurities acting as immune adjuvants, provoking alterations such as aggregation, particulates or ~~deamidation~~* ***immunogenic product variants.***	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
101	5	Comment: The reference for deamidated forms with an increased immunogenic potential should be provided. It is our understanding that endogenous human antibodies are also deamidated and that these forms should not necessarily be more immunogenic than non-deamidated antibodies. Proposed change (if any): Add reference or delete	Comment accepted.
102	7	Comment: There is a grammatical error. Text says "enforce" when it should say "increase". Proposed change (if any): Please change text to replace "enforce" with "increase"	Comment accepted.
102	11	Comment: There is a grammatical error. Text says "enforce" when it should say "increase". Proposed change (if any): Please change text to replace "enforce" with "increase".	
103-105	7	Comment: The glycosylation pattern, its similarity to human patterns, and changes especially during manufacturing, could raise an immune response and therefore, should be monitored. However there is no evidence that this	Comment not accepted. There is less experience with novel expression systems, so

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		depends on whether an expression system is novel or a-typical. Also typical expression systems may change over time. Proposed change (if any): Please re-phrase	such products need to be treated with care.
103-105	11	Comment: The glycosylation pattern, its similarity to human patterns, and changes especially during manufacturing, could raise an immune response and therefore, should be monitored. However there is no evidence that this depends on whether an expression system is novel or a-typical. Also typical expression systems may change over time. Proposed change (if any): Please re-phrase.	Comment not accepted. There is less experience with novel expression systems so such products need to be treated with care
108	7	Comment: Patient related factors may influence immunogenicity since they are known to do so. Proposed change (if any): Replace "may" by "are known to", resulting in "Patient related factors are known to influence immunogenicity...."	Comment accepted.
108-109	5	Comment: Major histocompatibility = human leukocyte antigen: just use HLA for humans	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Patient related factors may influence immunogenicity e.g., differences in human leukocyte antigen alleles among recipients...	
108-109	7	Comment: There is a spelling error - Histocompatibility It would be good to refer to the commonly used terms "MHC" and " HLA". In addition, there is currently redundancy: use either MHC or HLA. Proposed change (if any): Please correct the spelling error and consider using the terms "MHC" or " HLA".	Comment accepted.
108-109	11	Comment: There is a spelling error: correct spelling is Histocompatibility It would be good to refer to the commonly used terms "MHC" and " HLA". In addition, there is currently redundancy: use either MHC or HLA. Proposed change (if any): Please correct the spelling error and consider using the terms "MHC" or " HLA".	Comment accepted.
108-118	1	Comment: Also in this paragraph facts and assumptions are mixed. Proposed change (if any):	Comment not accepted. This comment is not well understood.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Separate facts from fiction	
108-118	5	Comment: In our opinion also in this paragraph facts and assumptions are mixed. Proposed change (if any): We would propose to clearly indicate (e.g. by reference to literature) which factors are proven to influence immunogenicity and to discriminate them from the ones that are rather based on speculation	Comment not accepted. References are not included in a regulatory guideline.
108-118	6	Comment: major histocompatibility is equal to human leukocyte antigen: just use HLA for humans Proposed change (if any): Change the sentence "Patient related factors may influence immunogenicity e.g., differences in major histocompatability and human leukocyte antigen alleles among recipients..." into "Patient related factors may influence immunogenicity e.g., differences in human leukocyte antigen alleles among recipients..."	Comment accepted.
111-114	7	Comment: It is written that immunogenicity for mAbs can be age related, as in RA vs. in juvenile arthritis. Please provide the source/ a literature citation for this observation.	Comment not accepted. This can be found in the EPAR for infliximab. References are not included in regulatory guidelines.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
111-114	11	Comments: It is written that immunogenicity for mAbs can be age related, as in RA vs. in juvenile arthritis. Please provide the source/ a literature citation for this observation.	Comment not accepted. This can be found in the EPAR for infliximab. References are not included in regulatory guidelines.
115-116	7	Comment: It is stated that "previous exposure to similar or related monoclonal antibodies can also influence immunogenicity". Please specify the background of this statement/ provide a literature reference	Comment not accepted. References are not included in a regulatory guideline.
115-116	11	Comment: It is stated that "previous exposure to similar or related monoclonal antibodies can also influence immunogenicity". Please specify the background of this statement/ provide a literature reference	Comment not accepted. References are not included in a regulatory guideline.
116-118	4	Comment: To sentence "Therapeutic antibodies used in a repeated dosing scheme or with intermittent dosing scheme changes have a higher likelihood to induce immunogenicity than single use mAbs." Actually continuous vs intermittent dosing is less immunogenic. Of course with a single-dose, immunogenicity will not be noticed unless an additional dosing at a later time point is needed, where the immune reaction could be very strong. Increased dosing levels may further reduce immunogenicity by the induction of tolerance.	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): *"Therapeutic antibodies used ~~in a repeated dosing scheme or~~ with intermittent dosing scheme* ***~~changes~~*** *have a higher likelihood to induce immunogenicity than* ***~~single use mAbs~~ when used in a scheduled and repeated dosing scheme. Increased dosing levels might reduce immunogenicity by the induction of tolerance."***	
116-118	5	Comment: In our opinion the sentence "Therapeutic antibodies used in a repeated dosing scheme or with intermittent dosing scheme changes have a higher likelihood to induce immunogenicity than single use mAbs." is not correct. Actually continuous dosing is less immunogenic than intermittent dosing. It is common sense that in most cases an immune response after a single-dose will not be noticed unless an additional dosing at a later time point is needed, where immune reaction could be very strong. Continuous dosing might further reduce immunogenicity by the induction of tolerance. Proposed change (if any): Please modify, e.g. as follows: "Therapeutic antibodies used with intermittent dosing schemes have a higher likelihood to induce immunogenicity than when used in a scheduled and repeated dosing scheme ~~than single use mAbs~~. Continuous dosing might reduce immunogenicity by the	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		induction of tolerance.”	
125-126	8	**Comment:** If section 5 is retained, the title should clearly explain when to consider these approaches. **Proposed change:** “Approaches which may be helpful in predicting and reducing the **development of unwanted anti-drug antibodies**.” The following should also be noted in the body of the text for this section: “**Predictions and de-immunization procedures are performed early in drug development and not usually during clinical trials**.”	Comment accepted..
125-137	9	Comment: This intended guidance in this section (on approaches which may be helpful in predicting and reducing the unwanted immunogenicity of mAbs) should be clarified. Beyond mentioning that both in silico and in vitro assays is the EMA offering any guidance on whether these methods are expected to be employed? We note that these method have not yet been fully clinically validated and therefore the text in the original immunogenicity guidance may be more appropriate. In addition with regards to the comments about T regulatory and T helper epitopes, we note there is currently very little scientific information on how T regulatory and helper cell epitopes differ. The guidance provided in the general immunogenicity guidance would be more appropriate for use here.	Comment not accepted. Text was modified to clarify when predictive methods are most likely to be used. The current text does not state that these procedures are mandatory. Further text changes are not deemed necessary.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change: Delete "In-silico modelling may help to identify T-cell epitopes but does not predict whether immunogenicity will occur. Confirmation/identification of T-cell epitopes using in-vitro cell based assays has been refined and is often applied to therapeutic mAbs. The relatively large size of the mAb molecule makes it likely that each molecule will contain several such epitopes. Both T-helper and T-regulatory epitopes have apparently been identified on mAbs." Replace with: "Non-clinical studies aiming at predicting immunogenicity in humans are normally not required. However, ongoing consideration should be given to the use of emerging technologies (novel *in vivo*, *in vitro* and *in silico* models), which might be used as tools." Delete "Deletion of T-helper epitopes may result in reduced immunogenicity, whereas the reverse would be the case for deletion of T-regulatory epitopes."	Comment accepted. The text has been ammended to state 'Non-clinical studies aiming at predicting immunogenicity in humans are normally not required.'
125-137	3	Comment: This section addresses the assessment of the intrinsic risk of immunogenicity due to the presence of T-helper epitopes in the primary amino acid sequence. It is important to emphasise to the Applicant that it is <u>invariably</u> helpful to understand the relative immunogenic potential, based on *in silico* and *in vitro* tools, of a monoclonal antibody for: • justification of the chosen amino acid sequence;	Comment partly accepted. The text was amended to include this.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		and • explanation of expected anti-drug antibody responses. Unfortunately, these points are not clear from the present text, which states what can be done without really explaining how the information should be used to facilitate the interpretation of the clinical observations. Whilst the design and selection of the mAb is, indeed, the responsibility of the applicant, information relating to intrinsic immunogenic potential could be very helpful in explaining why a product does induce host anti-drug antibodies. Whether these anti-drug antibodies are of clinical significance would be established by reference to the clinical observations. Nevertheless, without making the application of *in silico* and *in vitro* analyses obligatory, it is the regulator's role actively to encourage Applicant's to generate data that provides a sound basis for understanding the relative probability that a particular molecular entity could induce a host immune response. Proposed change (if any): Indicate that, whilst not obligatory, knowledge gained about the intrinsic immunogenicity of the primary amino acid sequence can be very helpful to (i) justify molecular engineering approach; and (ii) explain the basis of anti-drug antibody signals observed in clinical	Comment not accepted. The current level of detail of this part of the guideline is considered appropriate. Comment not accepted. The current level of detail of this part of the guideline is considered appropriate.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		studies. Accordingly, the application of *in silico* and/or *in vitro* techniques often provides information that is instructive to the immunogenicity risk assessment for monoclonal antibody products.	
125-137	4	Comment: This section describes one strategy out of many of how a manufacturer can develop a low immunogenic mAb. As with other strategies, the described one is discussed very controversial among experts and is therefore not ready to be included in the guideline. Overall, we believe that such a description of how to develop a mAb is up to the manufacturer and should not be part of an EMA guideline. Proposed change (if any): Delete Line 125-137 ~~**5. Approaches which may be helpful in predicting and reducing the unwanted immunogenicity of mAbs.**~~ ~~The design and selection of the mAb is the responsibility of the applicant. In-Vitro approaches with the aim of predicting immunogenicity have been developed (see general guidelines). In-silico modelling may help to identify T-cell epitopes but does not predict~~	Comment not accepted. This comment relates to biosimilars, which are not the subject of this guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		~~whether immunogenicity will occur.~~ ~~Confirmation/identification of T-cell epitopes using in-vitro cell based assays has been refined and may be applied to therapeutic mAbs. The relatively large size of the mAb molecule makes it likely that each molecule will contain several such epitopes. Both T-helper and T-regulatory epitopes appear to be present on mAbs, based on in-silico model.~~ ~~Various strategies for reducing the immonuogenicity of mAb therapeutics are currently being considered. These involve protocols for induction of tolerance to the mAb or 'de-immunizing' the mAb by deletion of relevant T-cell epitopes. Deletion of T-helper epitopes may result in reduced immunogenicity, whereas the reverse would be the case for deletion of T-regulatory epitopes.~~ Alternatively if the above is not acceptable, we propose the following changes to this section: Adding the following as a new paragraph 3 to this section: ***"While identification and deletion of T-cell epitopes may prove useful for reducing the immune response, it is generally a more relevant exercise for new biotherapeutic proteins. This technology has less application for comparability***	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		exercises where the protein sequence is identical to previous versions of the molecule, e.g., process changes for existing products and biosimilars."	
125-137	11	Comments: The scope of the guideline is described as guidance for preparation of the MAA (see line 72). Prediction of immunogenicity and deimmunisation approaches are relevant for the discovery and selection of the active compound. Therefore these aspects are irrelevant in the final development stage and the application should be guided by the actual immunogenicity data available. In our opinion, in silico and in vitro immunogenicity prediction technologies are too nascent to be recommended for general use, as there is little clinical validation data showing that these approaches really work. Therefore these approaches should not be recommended for use on a routine basis at the present time. Presence, relevance and impact of regulatory T cell epitopes are still a matter of controversial scientific discussion in many aspects. Please delete the word T-regulatory or specify that scientific confirmation is still lacking Proposed change (if any): Minimize references to such approaches or eliminate Section 5 from the guidance.	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
127-137	1	Comment: There is no evidence that any of these approaches are clinically relevant Proposed change (if any): Delete this section on predicting and reducing	Comment not accepted. The text does not state that these approaches are clinically relevant.
127-137	6	Comment: Too strong focus on T-regulatory cells. There is not enough data in the public domain to support the statement made in the last sentence of the second paragraph. It has been shown that the same epitope can activate T-regulatory cells and effector T cells alike (e.g. Fourcade et al. Journal of Immunol, 2010). This statement is based on publications for just one lab which also has commercial background. Although it is well appreciated that up-to-date science is mentioned in guidelines, statements have to be unbiased. Proposed change (if any): Please remove the last sentence ("Deletion of T-helper epitopes may result in reduced immunogenicity, whereas the reverse would be the case for deletion of T-regulatory epitopes") of the second paragraph.	Comment partly accepted. The text states that existing data are limited for Treg epitope recognition.
125-137	7	Comment: In silico as well as cellular approaches to define immunogenic epitopes of a protein are scientifically interesting tools, however, their predictive value for	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		immune reactions in the clinical situation has not been convincingly shown. Thus, they may be used for exploratory screening investigations but should not be a mandatory part of a safety evaluation. It should be mentioned that so far no method has proven generally predictive for human immunogenicity. Proposed change (if any): The entire paragraph 5 of the proposed guideline should be deleted. If section 5 is retained the title and text should clearly reflect the exploratory nature of the approaches described and specify when these approaches should be considered. "Approaches which may be helpful in predicting and reducing the development of ATA" The following should also be noted in the body of the text for this section: "Predictions and de-immunization procedures are performed early in drug development and not usually during clinical trials." Include references as justification or mention the immature stage of the technologies.	The guideline does not state that these procedures are mandatory. Further text changes are not deemed necessary.
125-137	10	Comment: The section on 'approaches which may be helpful in predicting and reducing the unwanted immunogenicity of mAbs' should be clarified with regards to the guidance, if any, being provided on using these methods as a regulatory expectation since the approaches discussed have not yet been clinically validated. There is currently very little scientific	The guideline does not state that these procedures are mandatory. Further text changes are not deemed necessary.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		information on how T-regulatory and -helper cell epitopes differ. Therefore, the current guidance provided in the general immunogenicity guidance would be more appropriate for use here. Proposed change: Delete "In-silico modelling may help to identify T-cell epitopes but does not predict whether immunogenicity will occur. Confirmation/identification of T-cell epitopes using in-vitro cell based assays has been refined and is often applied to therapeutic mAbs. The relatively large size of the mAb molecule makes it likely that each molecule will contain several such epitopes. Both T-helper and T-regulatory epitopes have apparently been identified on mAbs." Add "Non-clinical studies aiming at predicting immunogenicity in humans are normally not required. However, ongoing consideration should be given to the use of emerging technologies (novel *in vivo*, *in vitro* and *in silico* models), which might be used as tools." Delete "Deletion of T-helper epitopes may result in reduced immunogenicity, whereas the reverse would be the case for deletion of T-regulatory epitopes."	
129-130	7	Comment: The in silico approaches do not technically model T cell epitope identification, but rather predict binding of a peptide sequence to a particular HLA/MHC molecule. The key amino acids identified in these approaches often do not take into consideration epitopic residues,	Comment not accepted.

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		but rather focus on agretopic amino acids. While binding of peptide to MHC is an essential first step in T cell recognition, the ability of a TCR to recognize a particular peptide/MHC complex or for that particular TCR-bearing T cell to be present in a repertoire following thymic education is beyond the current scope of these methods. Current in silico approaches do not distinguish the potential influence of tolerance (central or peripheral) and how this influences or limits these data Proposed change (if any): Replace with "In silico modelling may help to identify peptide/MHC complexes, however, this does not predict whether immunogenicity will occur. Confirmation of T cell activation using in vitro binding or cell based assays is recommended. In addition, the potential influence of tolerance (central or peripheral) should be taken into account."	The proposal is too detailed and does not add anything significant to the existing text.
130-133	4	Comment: Testing for T-cell epitopes is still a relatively new technology for purposes of reducing the immunogenicity potential of a mAb, so stating that it is "often" applied to therapeutic mAbs is somewhat misleading. Proposed change (if any): *Confirmation/ identification of T-cell epitopes using cell*	Comment not accepted. mAbs are often selected using these techniques.

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		based assays has been refined and ~~is often~~ ***may be*** *applied to therapeutic mAbs. The relatively large size of the mAb molecule makes it likely that each molecule will contain several such epitopes. Both T-helper and T-regulatory epitopes* ***appear to be present*** *~~have apparently been identified~~ on mAbs* ***based on in-silico modeling and in-vitro testing.***	
130-137	5	Comment: It should be emphasised that so far no method has proven generally predictive for human immunogenicity. In addition we are of the opinion that the sentence "Deletion of T-helper epitopes may result in reduced immunogenicity, whereas the reverse would be the case for deletion of T-regulatory epitopes" is based on publications of a single lab which also has commercial background. Although it is well appreciated that up-to-date science is mentioned in guidelines, statements have to be unbiased. Proposed change (if any): Include references as justification or mention the immature stage of the technologies. Remove last sentence of second paragraph	Comment partly accepted. Text was modified. References are not included in regulatory guidelines.
132-133	7	Comment: The literature/data regarding epitopes recognized by T regulatory cells or the assays/methods used to identify these sequences is limited and should be specified in	Comment accepted.

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		the appropriate context. Proposed change (if any): Add " however , data supporting the identification of T regulatory cell epitopes is limited."	
134-137	5	Comment: 'De-immunizing' of mAB by deletion of T-cell epitopes is not applicable for biosimilar mABs as the identical amino acid sequence to the originator is a prerequisite. Proposed change (if any): Please include a sentence in this paragraph to reflect the above mentioned statement.	Comment not accepted. This is not deemed necessary.
135	4	Comment: The terminology 'de-immunizing' is misleading. What is meant is the reduction of the immunogenic potential of the mAb by deletion of relevant T-cell epitopes. Proposed change (if any): *"These involve protocols for induction of tolerance to the mAb or ~~'de-immunizing'~~* ***reduction of the immunogenic potential*** *of the mAb by deletion of relevant T-cell epitopes."*	Comment not accepted. The term 'de-immunizing' is widely used.
135	7	Comment: "De-immunizing" is a term used by a commercial vendor to refer to a service. Use of this term may have the appearance of endorsement of the vendor's service	Comment not accepted.

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		and should not be included in guidance document. Proposed change (if any): Replace with "These involve protocols for induction of tolerance or sequence engineering which modifies a protein sequence such that anchor residues important for binding to MHC are eliminated; thereby resulting in the reduction of elimination of T cell activation."	The term 'de-immunizing' is widely used.
138-184	9	Comment: These paragraphs contain information that appears to be pertinent to all protein biotherapeutics and is not unique to mAbs. The specific guidance with regard to mAbs is not clear Proposed change: Delete sections that are redundant with general guidance and reference that guidance. Clarify what clinical consequences are unique to mAbs and what actions should/need not be taken with regards to clinical evaluation of mAbs	Comment not accepted. Cannot identify 'redundant' passages.
138-184	10	Comment: These paragraphs contain information that appears to be pertinent to all protein biotherapeutics and not specifically unique to mAbs. As such, any specific guidance with regards to mAbs is not clear Proposed change: See general comment.	Comment not accepted. Cannot identify 'redundant' passages.
138-184	3	Comment:	Comment partly accepted. These sections had indeed too

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		This section addresses clinical consequences of immunogenicity of mAbs by presenting a number of general statements that do not represent helpful guidance to the Applicant for developing an effective strategy for particular products. For example: The statement "*It is important to note that not all induced antibodies are present in the serum, i.e. they may be present in various organs*" is not linked to any recommendation on how this observation might affect the clinical sampling strategy. The statement "*In some cases, low affinity IgM antibodies can be induced*" is not linked to a qualification that the detection of anti-mAb IgM ADA's is not generally associated with a clinically significant impact. The statement "*The availability of an appropriate IgE assay allows exclusion of these subjects with a positive result*" is not qualified by guidance recommending other tests, e.g. *ex-vivo* challenge of basophils by the drug product, that are often more sensitive and specific for detecting clinical risk of Type I hypersensitivity. It would be helpful to acknowledge that non-clinical evaluation of the incidence and magnitude of ADA responses in animals is likely to be of lower predictive value for the clinical setting in the case of a mAb product compared with other biopharmaceutical	much overlap with the general guideline on immunogenicity of therapeutic proteins. The entire clinical/risk part has been rewritten.

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		products, due to the confounding and often overwhelming influence of an anti-species response. Even if the host antibody response in non-human primates is less biased than in other animals, there is still insufficient relevance to justify specific measurement of the anti-idiotypic response in non-clinical studies. Determination of whether the host antibody response to a human mAb product is neutralising might have some relevance for interpretation of toxicology studies, but this would depend on the availability of suitably sensitive markers of the PD response. These non-clinical aspects should be addressed before moving onto the clinical situation. Proposed change (if any): On the basis that this section as presently written does not offer any "guidance" – as opposed to general statements on the phenomenon - for clinical evaluation of the immunogenicity of mAbs, it should be completely re-written to link specific recommendations for monitoring the rate of occurrence and severity of identified risk factors. References to measurement of antigen specific IgM and IgE might be supported as part of "for cause" investigations, but the current state of knowledge argues against routine measurement of these parameters. Most importantly, the guidance should address how the evaluation of the specificity of an ADA response to a	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		mAb should be prioritised according to the identified risk factors: for example, production of antibody fragments in microbial substrates should consider the possibility of signals associated with host cell-derived factors; mAb expression on non-human cell substrates should consider the risk of responses to non-human post-translational modifications; in most cases, the clinical impact of anti-idiotypic antibodies is likely to be more important than anti-allotypic antibodies – but, does the Applicant need to apply different assays to distinguish between them?; available evidence suggests that "persistent" ADA's are more clinical important than "transient" ADA's – but, how should the Applicant distinguish between these different types of ADA by optimising the timing of clinical sampling? Overall, if this guideline is to have any value, it needs to provide a risk-based prioritisation of the evaluation of immunogenicity in the clinical setting.	
138-184	5	Comment: The section is not specific for mAbs and as such already part of the general immunogenicity guidance (EMEA/CHMP/BMWP/14327/2006). Proposed change (if any): Delete section 6. If the section will be kept, please consider the following comments:	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
138-184	7	Comment:	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		The section is not specific for mAbs and as such already part of the general immunogenicity guidance. Proposed change (if any): Shorten section 6, and make reference to the general EMA immunogenicity guideline	The clinical and risk sections have been redrafted taking these and other comments into account.
138-184	11	Comment: The chapter 6. does not discuss specific considerations for mAb therapeutics. Please delete or change to mAb specific points.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
139	7	Comment: "The clinical consequences described following antibody development against mAbs include loss or reduction of efficacy, local reactions, serum sickness/immune complex-mediated disease, and major allergic reactions (e.g. urticaria, bronchospasm, bronchoconstriction)." This text implies that there are always several types of clinical sequelae to an ATA response, which is not the case in our experience. Proposed change (if any): "The clinical consequences…MAY include loss or reduction……immune complex-mediated disease, OR major allergic reactions"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
139	11	Comment: "The clinical consequences described following antibody development against mAbs include loss or reduction of efficacy, local reactions, serum sickness/immune	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		complex-mediated disease, and major allergic reactions (e.g. urticaria, bronchospasm, bronchoconstriction)." This text implies that there are always several types of clinical sequelae to an ATA response, which is not the case in our experience. Proposed change (if any): "The clinical consequences…MAY include loss or reduction……immune complex-mediated disease, OR major allergic reactions"	
141	10	Comment: The examples 'bronchospasm' and 'bronchoconstriction' may be seen as too similar or alike to some. Proposed change: 'urticaria, bronchospasm, *hypotension*'	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
144	7	Comment: Replace the words "this potential reaction" by "the consequences"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
144	11	Comment: Replace the words "this potential reaction" by "the consequences"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
145	8	**Comment:** The term "present" may not be the appropriate word here. **Proposed change:** We suggest "[...] not all induced antibodies are ~~present~~ **detectable** in the serum."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
145-151	6	Comment: Since mAb and ADA are both antibodies it is very important to be univocal. Proposed change (if any): In the second sentence "It is important during the clinical development to measure antibody levels, PK…" change "antibody levels" into "ADA". Make sure to be univocal throughout the guidance.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
145-151	11	Comment: Please describe the consequences for immunogenicity assessment; the analysis of ATAs in organs etc of humans is challenging or even impossible.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
146	7	Comment: It is unclear whether "antibody levels" means semi-quantitative assays or qualitative assays. Proposed change (if any): Replace with "It is important during clinical development to detect antibodies against mAbs and measure PK, PD markers, efficacy and safety simultaneously and over a period of repeated treatments." Comment: Since the chapter starts with a discussion on antibodies appearing in various tissues, it should be clarified that	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		during clinical development only serum antibodies specific for therapeutic antibodies or antibody-derived products have to be determined. Otherwise, please consider and discuss the consequences of the request regarding immunogenicity assessment, since analysis of ATAs in organs etc of humans is challenging or even impossible. Proposed change (if any): It is important during the clinical development to measure serum antibody levels.....	
146-147	8	**Comment: "**It is important during the clinical development to measure antibody levels, [...] over a period of repeated treatments." This statement suggests that Anti-Drug Antibody (ADA) would not be measured after single dose. A statement regarding the measurement of ADA after single dose should be added. A consideration should be made for mAbs with long circulating half-lives and exposures ranging from weeks to months. **Proposed change:** "It is important during the clinical development to measure antibody levels, PK, PD markers, efficacy and safety simultaneously and over a period of **single and** repeated treatments."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
146-147	7	Comment: "It is important during the clinical development to measure antibody levels, [...] over a period of repeated	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		treatments." This statement suggests that Anti-Drug Antibody (ADA) would not be measured after single dose. A statement regarding the measurement of ADA after single dose should be added. A consideration should be made for mAbs with long circulating half-lives and exposures ranging from weeks to months. Proposed change (if any): "It is important during the clinical development to measure antibody levels, PK, PD markers, efficacy and safety simultaneously and over a period of single and repeated treatments."	
150-151	7	Comment: "…Unexpected clinical observations (e.g., loss of efficacy or considerable differences in PK) could be the result of undetected antibodies and should be further investigated." Changes in PK over time could be due to other confounding factors. For example, disease status, concomitant medications, target-mediated drug disposition, as mentioned in the guidelines. The word "unexpected" is subjective. The same clinical observations may be explained in other ways, and thus would no longer be "unexpected". Immunogenicity and its possible contribution to clinical observations should always be in the scope of assessment. Proposed change (if any):	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Suggest removing "unexpected". "…Clinical observations (e.g., loss of efficacy or considerable differences in PK) could be the result of undetected antibodies and should be further investigated."	
150-151	11	Comment: "…Unexpected clinical observations (e.g., loss of efficacy or considerable differences in PK) could be the result of undetected antibodies and should be further investigated." Changes in PK over time could be due to other confounding factors. For example, disease status, concomitant medications, target-mediated drug disposition, as mentioned in the guidelines. The word "unexpected" is subjective. The same clinical observations may be explained in other ways, and thus would no longer be "unexpected". Immunogenicity and its possible contribution to clinical observations should always be in the scope of assessment. Proposed change (if any): Suggest to remove "unexpected".	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		"…Clinical observations (e.g., loss of efficacy or considerable differences in PK) could be the result of undetected antibodies and should be further investigated."	
152	7	Comment: The statement "Treatment with mAb can lead to the development of any class of immunoglobulin"….. needs clarification.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
152	11	Comment: The statement "Treatment with mAb can lead to the development of any class of immunoglobulin"….. needs clarification. It may be more appropriate to say "…development of an ATA response based upon any class of immunoglobulin…" Proposed change (if any): Please modify text to say "…development of an ATA response based upon any class of immunoglobulin	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
152-157	6	Comment: Does this mean that we always need an IgM specific assay?	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Please include more specific information on the appropriate tests needed to address this problem.	
153	4	Comment: The role of IgM Abs for ("long term") immunogenicity has to be clarified as a clinically relevant immune response would result in IgG class switch. Proposed change (if any): Please consider the above comment in the guideline text.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
153-154	7	Comment: Anti-Drug Antibodies (ADA) can interfere with the PK assay, producing results that suggest that the drug is eliminated from the system, while the drug may be present but may not be detected due to ADA interference. Proposed change (if any): We suggest revising the following sentence "Antibodies can reduce the PK, PD and efficacy and can result in neutralisation of the mAb." to include the consideration for an "apparent reduction of PK" due to assay interference by ADA. Please modify the text to say "can affect the PK, PD..."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
153-154	8	**Comment:** Anti-Drug Antibodies (ADA) can interfere with the PK assay, producing false-negative results that suggest that the drug is eliminated from the system.	Comment partly accepted. The clinical and risk sections have been redrafted taking these

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		The drug may be present, but may not be detected due to ADA interference. **Proposed change:** We suggest revising the following sentence, "Antibodies can reduce the exposure, PD effect and efficacy, and can result in neutralisation of the mAb," to include the consideration for an "apparent reduction of exposure to the mAb" due to assay interference by ADA.	and other comments into account.
153 - 154	11	Comment: "Antibodies can reduce the PK, PD and efficacy and can result in neutralisation of the mAb." It may be more appropriate to say "can affect the PK, PD..." ,than to say "can reduce...", since ATAs do not necessarily always reduce exposure. Proposed change (if any): Please modify text to say "can affect the PK, PD..."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
154-155	4	Comment: The ability to measure induced antibody in the serum is not only limited by the clearances of complexes but also by drug interference. Proposed change (if any): Please modify the sentence as follows: *"The ability to measure induced antibody in the serum… is limited by the clearances of complexes* ***or by interference with therapeutic mAb drug levels still present in the serum (i.e., drug tolerance) or by interference caused by disease related substances, agents, or features in the patient's***	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		serum such as rheumatoid factor, i.e. matrix effect."	
154-155	7	Comment: "The ability to measure induced antibody in the serum is limited by the clearance of complexes". Please specify the consequences of this statement	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
154-155	11	Comments: "The ability to measure induced antibody in the serum is limited by the clearance of complexes". Please specify the consequences of this statement	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
158	4	Comment: Regarding Lines 158-159, IgE testing should not be required for CHO lines (except as dictated by product specific attributes) despite the presence of non-human carbohydrate structure. Clinical experience with CHO is well established and IgE reactions are generally not concerning. Proposed change (if any): Recommend inserting a new sentence to read: *"IgE testing is generally not needed for CHO cell lines, despite the presence of non-human carbohydrate structures, owing to the large clinical experience with product produced in this cell line."*	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
158-161	9	Comment: Information on consequences if an antibody contains non-human carbohydrate structure could apply to a	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		large number of mAbs and any non-mAb biotherapeutics that are produced in non-human mammalian cells, although the carbohydrates have not been shown to be problematic for most of these. IgE testing should not be needed where the carbohydrates are not known to be associated with allergic reactions and no allergic reactions have been observed. Proposed change: Replace current text with: In some instances, IgE testing needs to be considered for patients e.g. if the mAb contains non-human carbohydrate structures associated with IgE responses or where the incidence of allergic reactions occurs on first administration or after ADA development.	
158-160	1	Comment: The predictive value for an allergic reaction of IgE testing in general is low. And there is no evidence at all for therapeutic proteins Proposed change (if any): Delete	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
158-162	8	**Comment:** We suggest leading into the IgE section with a statement regarding clinical consequences of IgE. We also suggest revising the IgE section with a statement similar to Line 163 for IgA: "IgA antibody testing may only be needed on a case-by-case basis."	Comment partly accepted. This was reworded.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change: "In some instances, mAb **product may induce IgE-mediated allergic reactions. Route of administration and host cell structural modifications of the mAb product are among the factors considered for potential induction of IgE. IgE antibody testing may only be needed on a case-by-case basis**."	
158-162	8	**Comment:** "In some instances, IgE testing needs to be considered for patients if the mAb contains non-human carbohydrate structures." The "non-human carbohydrate structures" seem to refer to a reported correlation of IgE from humans treated with cetuximab cross-reacting with beef or tick carbohydrate structures. The patients had pre-existing IgE, prior to treatment with cetuximab. Therefore, the testing would only be useful as a pre-treatment screen when one knew what to screen for. The issue is that "non-human carbohydrate structures" can also originate from CHO and NS0 cell lines, and we do not believe that IgE screening should be required for all products derived from non-human cell lines. **Proposed change:** We suggest deleting the following sentence: "~~In some instances, IgE testing needs to be considered for patients if the mAb contains non-human carbohydrate structures~~." The fact that host cell alterations may be a factor can be included in the sentence suggested in the above comment on Lines 158-162 ("Route of administration and host cell	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		structural modifications of the mAb product are among the factors considered for potential induction of IgE.")	
158-159	11	Comment: "In some instances, IgE testing needs to be considered for patients if the mAb contains non-human carbohydrate structures." To our knowledge, thus far, it has primarily been the non-human carbohydrates expressed on NS0 mouse derived Cetuximab that have been problematic. Many mAbs are produced in CHO (hamster) cell lines and contain non-human carbohydrates without any similar problem. Proposed change (if any): IgE testing should be considered when clinical evidence of allergic/hypersensitivity reactions is observed.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
158 -159	11	Comment: "In some instances, IgE testing needs to be considered for patients if the mAb contains non-human carbohydrate structures." This text would benefit from clarification, to specify that the IgE testing mentioned here refers to testing for drug-specific IgE (as opposed to testing for allergen-specific IgEs like pollen, ragweed). In addition, the presence of IgE does not necessarily imply an allergic response. Proposed change (if any): "Testing for IgE isotype ATAs may need to be considered." Specify cases when IgE assay would be required.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
159-160	11	Comment: "Another instance where development of IgE testing should be considered is where the incidence of allergic reactions is high on first administration during early clinical development of the product. The availability of an appropriate IgE assay allows exclusion of those subjects with a positive result." Subjects with pre-existing IgE antibodies to a related molecule, which can cross-react with the therapeutic mAb may have this problem (c.f. Cetuximab). Alternatively, subjects who have been exposed to a different type of protein therapeutic previously and who have developed an IgE type ATA response to this protein, which can cross-react with the therapeutic mAb may have this problem. It would be helpful to add a clear definition of what patient populations are considered high risk, as the tolerance for adverse events in oncology subjects may differ from that in immunology indications. In some cases, very low levels of antigen/allergen specific IgE can drive a profound systemic hypersensitivity response. It is not clear to us that IgE assays can be developed to have adequate sensitivity to be able to exclude the possibility of allergic reactions occurring in all hypersensitive subjects. In addition, immediate allergic-like reactions (upon "first administration" as named in the text) are mediated by antibody-independent factors, such as cytokines and complement, because antibodies have not yet been formed and may also depend on the	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		intended pharmacologic mechanism of the drug. Proposed change (if any): Please clarify the context here. In very rare cases, in particular when other more common factors for first-infusion reaction can be excluded, development of IgE tests should be considered to evaluate pre-existing IgE ATAs	
158-162	5	Comment: Almost all Abs contain non-human carbohydrates. Therefore the guideline should be clearer, e.g. by stating that IgE testing is required if the Ab contains carbohydrates known to play a role in allergic diseases such as food allergy. Proposed change (if any): Replace first sentence with: "In some instances, IgE testing is required if the Ab contains carbohydrates known to play a role in allergic diseases such as food allergy."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
158-162	6	Comment: • Please define appropriate IgE: does it mean a general or drug specific IgE assay? • Antibodies for clinical use may contain non-human carbohydrates. Therefore the guideline should be clearer, e.g. by stating that IgE testing is required if the Ab contains carbohydrates known to play a role in allergic	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		diseases such as food allergy. • The guidance seems vague on this topic. IgE testing is not always feasible since concentrations are mostly very low. Proposed change (if any): • Replace the first sentence "In some instances, IgE testing needs to be considered for patients if the mAb contains non-human carbohydrate structures" with: "In some instances, IgE testing is required if the Ab contains carbohydrates known to play a role in allergic diseases such as food allergy." • More general: Emphasize that clinical symptoms should be leading for the consideration to do IgE testing.	
158-162	7	Comments: "In some instances, IgE testing needs to be considered for patients if the mAb contains non-human carbohydrate structures." This text would benefit from clarification. It should be sspecified that the IgE testing mentioned here refers to testing for drug-specific IgE (as opposed to testing for allergen-specific IgEs like pollen, ragweed). We suggest leading into the IgE section with a	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		statement regarding clinical consequences of IgE. We also suggest revising the IgE section with a statement similar to Line 163 for IgA: "IgA antibody testing may only be needed on a case-by-case basis". In addition, to our knowledge, thus far, it has primarily been the non-human carbohydrates expressed on NS0 mouse derived Cetuximab that have been problematic. The patients had pre-existing IgE, prior to treatment with cetuximab. Therefore, the testing would only be useful as a pre-treatment screen when one knows what to screen for. Many mAbs are produced in CHO (hamster) cell lines and contain non-human carbohydrates without any similar problem. We do not believe that IgE screening should be required for all products derived from non-human cell lines. Provided the request for IgE assay is in the final guideline, please provide additional clarification regarding an appropriate IgE assay. Does the CHMP mean a general or drug specific IgE assay? It is not clear to us that IgE assays can be developed to have adequate sensitivity to be able to exclude the possibility of allergic reactions occurring in all hypersensitive subjects. Proposed change (if any): Please clarify the context here, and consider deleting the following sentence: "~~In some instances, IgE testing needs to be considered for patients if the mAb contains~~	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		~~non-human carbohydrate structures~~" or specify cases when IgE assay would be required. IgE testing should be considered when clinical evidence of allergic/hypersensitivity reactions is observed, and is needed on a case-by-case basis only. In very rare cases, in particular when other more common factors for first-infusion reaction can be excluded, development of IgE tests should be considered to evaluate IgE ATAs Please add that the exclusion of IgE positive subjects from therapy or termination of treatment should only be done if clinical relevance of IgE and the occurrence of an adverse event has been shown (compare statement to lines 182-184)	
158-161	10	Comment: IgE testing should not be needed if the carbohydrates are not known to be associated with allergic reactions and no allergic reactions have been observed. Proposed change: "In some instances, IgE testing needs to be considered for patients *e.g.* if the mAb contains non-human carbohydrate structures *associated with IgE responses or where the incidence of allergic reactions occurs on first administration or after ADA development*."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
161-162	5	Comment: Regarding the sentence: "IgE assay allows exclusion of subjects with a positive result": In our opinion IgE levels are not necessarily directly correlated to allergy.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		IgE assays can in our view only be of added value to explain whether clinically observed hypersensitivity reactions were caused by IgE or by other factors (e.g. complement). This might affect the decision on re-administering the drug. Proposed change (if any): Reconsider the sentence. There might be also alternatives to IgE testing in order to reduce the risk of a patient to develop allergy before entering a clinical trial.	
161-162	11	Comment: Please add that the exclusion of IgE positive subjects from therapy or termination of treatment should only be done if clinical relevance of IgE and the occurrence of an adverse event has been shown (compare statement to lines 182-184)	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
163	7	Comment: It is not clear from this guidance what the clinical utility of testing for IgA ATAs is. Proposed change (if any): Please clarify the clinical utility of this testing, and if there are specific clinical sequelae that are driven solely by IgAs, please provide literature citations.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
163	11	Comment: It is not clear from this guidance what the clinical utility of testing for IgA ATAs is.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Please clarify the clinical utility of this testing, and if there are specific clinical sequelae that are driven solely by IgAs, please provide literature citations.	other comments into account.
163-168	1	Comment: There is no evidence that IgA testing is ever useful Proposed change (if any): Delete	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
163-168	5	Comment: In our opinion there is no evidence that IgA testing is ever useful. Proposed change (if any): Delete paragraph	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
169	4	Comment: please specify the immune response as „unwanted" Proposed change (if any): „In many cases, the incidence of **unwanted** immune response is too low…"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
169-181	8	**Comment:** The intent to recommend post-marketing surveillance of clinical signs suggestive of ADA-mediated reactions should be clarified. **Proposed change:** We suggest combining Lines 169-172 with Lines 179 -181: "In many cases, the	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		incidence of immune response is too low to be fully identified during Phase III clinical studies **and antibodies against mAbs are rarely monitored in clinical practice. In these situations, it is** ~~T~~therefore **important to have an adequately organised** ~~systematic~~ post-authorisation monitoring **process** ~~may be necessary and should be adequately organised~~ to capture clinical signs that could be related to immunogenicity. ~~The involvement of antibodies in this should be established by conducting appropriate assays.~~ **If an anti-drug antibody-related issue is identified, appropriate assay to characterise the immune response should be performed.** [...] ~~Because detection of antibodies against mAbs is rarely monitored in clinical practice, it is unclearother than in instances of obvious clinical evidence of one of the presentations listed above whether~~ the development of antibodies to mAbs has additional unrecognised consequences.	
169-181	7	Comment: The intent to recommend post-marketing surveillance of clinical signs suggestive of ADA-mediated reactions should be clarified. Proposed change (if any): We suggest combining Lines 169-172 with Lines 179 - 181: "In many cases, the incidence of immune response is too low to be fully identified during Phase III clinical studies and antibodies against mAbs are rarely monitored in clinical practice. It is ~~T~~therefore important to have an adequately organised ~~systematic~~ post-authorisation monitoring process ~~may be~~	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		~~necessary and should be adequately organised~~ to capture clinical signs that could be related to immunogenicity. ~~The involvement of antibodies in this should be established by conducting appropriate assays.~~ If an anti-drug antibody related issue is identified, appropriate assay to characterise the immune response should be performed. [...] ~~Because detection of antibodies against mAbs is rarely monitored in clinical practice, it is unclearother than in instances of obvious clinical evidence of one of the presentations listed above whether~~the development of antibodies to mAbs has additional unrecognised consequences.	
173-178	5	Comment: In our opinion allergy and serum sickness symptoms themselves are already sufficient to drive clinical decisions. It is questionable if determining that these symptoms were caused by detectable anti-drug antibodies would these decisions. The same holds true for lack in efficacy. Proposed change (if any): Reconsider	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
173-178	6	Comment: There is no information on how this post marketing observation is expected to be conducted.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Add that the post marketing measurement of ADA is lead by clinical observations, and that duration of this process is dependent on the risk and therefore discussed with regulatory bodies.	
174-176	9	Comment: The guidance provided is not clear with regard to whether there is a need for ADA assay in the post-marketing setting for every adverse event or loss of efficacy report that could be attributed to ADA, especially if the responses have already been characterized in clinical studies. Proposed change: Change to: In cases where adverse events or loss of efficacy follow administration of the implicated mAb, the effects may be attributed to an antibody response. Depending on the seriousness of the effects to patient safety, it may be necessary to confirm and characterize antibody induction.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
174-176	10	Comment: The guidance provided is not clear with regards to whether there is a need for an ADA assay in the post-marketing setting for every adverse event or loss of efficacy report that may be attributed to an ADA, especially if the reported response has already been characterized in clinical studies. Proposed change: "In cases where adverse events *or loss of efficacy* follow administration of the implicated	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		mAb, the reactions are *effects may be* due to an antibody response. *Depending on the seriousness of the effects to patient safety, it may be necessary to confirm and characterize antibody induction*."	
174-176	7	Comment: Some adverse events following mAb administration can reasonably be attributed to ATAs, but it should not be automatically assumed that all adverse events are ATA driven. Rather, it should be a reasoned assessment based on the symptomatology. Similarly, loss of efficacy can be due to reasons other than ATA, as this has been shown to occur with non-biologicals where no ATAs are to be found. Proposed change (if any): Please remove the assumption that ATAs always drive AEs, and indicate that an evidenced-based approach should be used.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
174-176	11	Comment: Some adverse events following mAb administration can reasonably be attributed to ATAs, but it should not be automatically assumed that all adverse events are ATA driven. Rather, it should be a reasoned assessment based on the symptomatology. Similarly, loss of efficacy can be due to reasons other than ATA, as this has been shown to occur with non-biologicals where no ATAs are to be found. Proposed change (if any): Please remove the	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		assumption that ATAs always drive AEs, and indicate that an evidenced-based approach should be used.	
175	4	Comment: Adverse events after mAb administration may be caused by anti-mAb antibodies, however, adverse events might also be caused by other factors. Proposed change (if any): Please change wording as follows: "In cases where …, the reactions ~~are~~ **may be** attributed to an antibody response."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
175	5	Comment: Adverse events after mAb administration may be caused by anti-mAb antibodies, however, adverse events might also be caused by other factors. Proposed change (if any): Please change wording as follows: "In cases where …, the reactions ~~are~~ may be attributed to an antibody response."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
175-178	8	**Comment:** The Industry current practice is to determine the extent of characterization of antibody induction based upon the risk assessment. Lines 175-178 seem to suggest that there would be no need to perform a risk assessment, and that any observation that suggests induction of immunogenicity, adverse events or loss of efficacy would require full characterization regardless of the perceived risk to patients. **Proposed change:** Please clarify the seriousness of	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		the development of unwanted immunogenicity as it pertains to mAbs.	
176	4	Comment: Loss or reduction of response does not automatically imply antibody formation. One needs to measure drug trough levels to ensure adequate dosing, as there is inter-individual variability as to the mAb and drug clearance. Proposed change (if any): Delete: ~~"The same rationale applies in cases where loss of efficacy is observed."~~ Replace with: ***"Anti-mAb antibodies might also be the reason for loss of efficacy."***	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
176	5	Comment: Loss or reduction of response does not automatically imply antibody formation. One needs to measure drug trough levels to ensure adequate dosing, as there is inter-individual variability as to the mAb and other drug clearance. Proposed change (if any): Delete the sentence "The same rationale applies in cases where loss of efficacy is observed." OR change the wording as follows: "Anti-mAb antibodies might also be the reason for loss	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		of efficacy.”	
176-178	7	Comment: Events that occur following marketing authorization are typically observed outside the auspices of a formal clinical trial. As stated in lines 179-181, this is rarely done in routine clinical practice. Proposed change (if any): Please clarify- is the guidance primarily intended to cover therapeutic mAb development, or does it extend into clinical practice once the therapeutic is approved as well?	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
176-178	11	Comment: Events that occur following marketing authorization are typically observed outside the auspices of a formal clinical trial. Is the EMA suggesting that an ATA assay be made available by sponsors to any requesting physician after each biotherapeutic is approved and in use in the public domain? As stated in lines 179-181, this is rarely done in routine clinical practice. Proposed change (if any): Please clarify--is this intended guidance primarily intended to cover therapeutic mAb development, or does it extend into clinical practice once the therapeutic is approved as well?	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
179-181	1	Comment: What could these unknown consequences be? Proposed change (if any):	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Delete	
179-181	4	Comment: The sentence is not meaningful: Proposed change: Delete Line 179-181 Because detection of antibodies against mAbs is rarely monitored in clinical practice, it is unclear – other than in instances of obvious clinical evidence of one of the presentations listed above whether the development of antibodies to mAbs has additional unrecognised consequences.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
182	9	Comment: The guidance provided with respect to detection of antibodies in low dose cohorts (detection does not necessarily justify termination of treatment) should apply to all dose levels. Proposed change: Delete "in low dose cohorts"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
182	10	Comment: The guidance provided with respect to detection of antibodies in low dose cohorts ('detection…does not necessarily justify termination of treatment') should apply to all dose levels. Proposed change: Delete "in low dose cohorts".	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
182-184	1	Comment: Stopping treatment is always a clinical judgement Proposed change (if any): Delete	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
182-184	6	Comment: In the phrase "in low dose cohorts...termination of treatment", does " treatment" mean "dose escalation to higher dose cohorts" or does it refer to the individual patient/subject? In case antibodies are detected, reduced efficacy is likely. Doesn´t it make sense to stop treatment of a patient then even without clinical findings (but for example altered PK/PD)?	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
182-184	7	Comment: The intentions of this statement are unclear. The decision to terminate treatment should be based on clinical findings in all subjects and not just those receiving low doses. Proposed change (if any): We suggest expanding the statement to clarify the point to be made. Remove the phrase "in low dose cohorts".	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
182-184	8	**Comment:** The intentions of this statement are unclear.	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change: We suggest removing or expanding upon to clarify the point to be made.	The clinical and risk sections have been redrafted taking these and other comments into account.
182-184	11	Comment: "Detection of antibodies in low dose cohorts does not necessarily justify termination of treatment. The need to terminate treatment because of antibody formation can only be assessed in combination with clinical findings and requires careful assessment and monitoring." We agree that antibody impact on mAb PK at lower doses may not be extrapolated to higher dose levels. It is not clear why detection of antibodies at low dose levels is singled out. It seems that this paragraph is applicable to all dose levels. Proposed change (if any): Suggest to remove "in low dose cohorts".	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
185-186	7	Comment: Title should be revised to be consistent with verbiage across the document. Proposed change (if any): "~~Problems experienced with screening and confirmatory assays used in assessing immunogenicity of mAbs~~" "Considerations for detection and confirmation of antibodies to mAbs"	Comment not accepted. This section deals primarily with 'problems' and the title should reflect this.
185-186	8	**Comment:** Title should be revised to be consistent with verbiage across the document. **Proposed change**: "~~Problems experienced with~~	Comment not accepted. This section deals primarily with 'problems' and the title should reflect this.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		~~screening and confirmatory assays used in assessing immunogenicity of mAbs~~" "**Considerations for detection and confirmation of antibodies to mAbs**"	
185-213	3	Comment: Although this section presents important considerations regarding the advantages and disadvantages of different technical approaches, the text does not provide the reader with clear messages that explain the optimal bioanalytical approach for evaluation of ADA's to mAb products. Substantial accumulated experience indicates that although alternate approaches might be adopted, there are three methods that should be preferred for mAb products, namely: • Bridging ELISA to detect divalent ADA's • Radioimmunoprecipitation using labelled mAb • Surface Plasmon Resonance. The guideline should indicate that it is the responsibility of the Applicant to justify the suitability of the chosen approach, taking into full consideration the limitations of the respective methods. Arguably, the guideline should emphasise those aspects of the validation of assay suitability that most often lead to regulatory questions:	Comment partly accepted. All this is in the guideline. Added to text.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		• Threshold for drug interference • Detection of endogenous target antigen in bridging format • Interference by heterophilic antibodies, including Rheumatoid Factors • Capacity to measure ADA of different immunoglobulin class/subclass. The guideline should explain that the specificity and sensitivity of the assay should be adequate to detect clinically significant levels of ADA's, as judged by correlation with PK, PD, efficacy and safety indices. For example, in the case of anti-TNF mAbs, measurement of trough concentrations of the mAb product may provide a more reliable index of clinical significance than the level of ADA's measured in a quasi-quantitative assay (Aarden L *et al*; Curr Opin Immunol 2008, 20, 431-435). Proposed change (if any): Substantial accumulated experience indicates that although alternate approaches might be adopted, there are three methods that should be preferred for mAb products, namely: • Bridging ELISA to detect divalent ADA's • Radioimmunoprecipitation using labelled mAb • Surface Plasmon Resonance.	This was added to the guideline text.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		It is the responsibility of the Applicant to justify the suitability of the chosen approach, taking into full consideration the limitations of the respective methods. The validation of the suitability of the chosen method should follow the recommendations given in the general guideline, but should give particular attention to the following aspects: • Threshold for drug interference • Detection of endogenous target antigen in bridging format • Interference by heterophilic antibodies, including Rheumatoid Factors • Capacity to measure ADA of different immunoglobulin class/subclass. The specificity and sensitivity of the assay should be adequate to detect clinically significant levels of ADA's, as judged by correlation with PK, PD, efficacy and safety indices. For example, in the case of anti-TNF mAbs, measurement of trough concentrations of the mAb product may provide a more reliable index of clinical significance than the level of ADA's measured in a quasi-quantitative assay (Aarden L *et al*; Curr Opin Immunol 2008, 20, 431-435).	
185-213	7	Comment: Section 7 attempts to describe some of the challenges associated with screening and confirmatory	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		immunogenicity assays. This section could be truncated. It might be useful to refer to the available industry "White papers" that describe best practices with regard to these challenges. Proposed change (if any): Consider truncating this section and citing the above mentioned papers as a good source of information on ATA assay design, development & validation.	Comment not accepted. References are not included in regulatory guidelines. It would be very difficult to truncate and therefore this is not an acceptable option. References are not included in regulatory guidelines.
185-213	11	Comment: Section 7 attempts to describe some of the challenges associated with screening and confirmatory immunogenicity assays. This section could be truncated. It might be useful to refer to the available industry "White papers" that describe best practices with regard to these challenges. Proposed change (if any): Consider truncating this section and citing the above mentioned papers as a good source of information on ATA assay design, development & validation.	It would be very difficult to truncate and therefore this is not an acceptable option. References are not included in regulatory guidelines.
188-207	5	Comment: In general we are of the opinion that assay formats should not be described in a guideline. Proposed change (if any): Remove complete section since a discussion of available	Comment not accepted This section is necessary and cannot be deleted. General characteristics of assays do not change / improve rapidly.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		methods is not required in a guideline especially since methods change / improve rapidly. In case the section will not be deleted the following points should be considered: A number of ADA assays for mAbs utilizing Protein G format were successfully validated in pre-clinical and clinical settings (Biacore based-Protein G chip ADA assays). Bridging assays usually provide sufficient sensitivity and do not require significantly higher development efforts than other immunoassay formats. Due to a sequential analysis of samples, SPR has a lower throughput than plate based methods and therefore SPR can not be considered faster than other immunoassays.	This proposal is too detailed but most of this information is in the guideline. The guideline does not state that SPR is faster than other methods.
188-207	6	Comment: • Does the statement "It also will not efficiently detect the IgG_4 antibodies" imply the requirement of a validated IgG4 assay? • Is evaluation of antibody titers expected? • General comment to complete section: Too many opinions. The different assay formats should not be discussed in a guideline. • Regarding the statement "Another approach is to use a Surface Plasmon Resonance (SPR) ...	Comment partly accepted. No. This is discussed in the general guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		It is a real-time procedure and is therefore fast ...” the following: SPR has due to a sequential analysis of samples lower throughput than plate based methods and therefore SPR is not faster than immuno-assays. • This are very general considerations and are not specifically therapeutic Mab topics. Added value of this is questionable. Proposed change (if any): • Consider to remove complete section since a discussion of available methods is not required in a guideline especially since methods change / improve rapidly. In case the section will not be deleted the following points should be considered: • A number of ADA assays utilizing Protein G format are successfully validated clinically and preclinically (Biacore based-Protein G chip ADA assays) • Bridging assays usually provide sufficient sensitivity and does not require more significant development effort than other immunoassay formats. • Change the text “It is a real-time procedure … other methods” to: “It is a real-time procedure	The guideline does not state that SPR is faster than other methods. This section is necessary and cannot be deleted. General

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		and also detects rapidly dissociating antibodies which can be missed by other methods" • Exclude the part "Many standard assay formats involve the use of anti-immunoglobulin reagents such as antibodies against immunoglobulins, protein A or protein G, but these are inappropriate for use in detecting antibodies against mAbs as they very often bind to the product itself" completely. Exclude the part "However, this procedure may be less sensitive than other immunoassay methods and can require significant development effort to produce a suitable assay."completely.	characteristics of assays do not change / improve rapidly
194-195	7	Comment: Protein A and Protein G are useful reagents for confirmatory assays. Proposed change (if any): Add "however, Protein A and Protein G may be appropriate in confirmatory assays to demonstrate that the positive response is due to an immunoglobulin."	This was added to the guideline text.
197	4	Comment: On Line 197, the sentence needs to be clarified to apply to mAbs. Proposed change (if any):	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		*"Therefore, different assay approaches have to be adopted/developed **for mAbs**".*	
198-202	4	Comment: ECL assays are missing. ECL is another bridging format commonly used and should be mentioned here and noted that it does not suffer the sensitivity issues of ELISA. Proposed change (if any): *A common approach is to use a "bridging" **format (e.g., ELISA or ECL)** which does not require anti-immunoglobulin reagents and so can be directly applied to studies with mAbs. However, ~~this procedure~~ the ELISA format may be less sensitive than other immunoassay methods and can require significant development effort to produce a suitable assay.*	Comment accepted.
199-200	9	Comment: There is no evidence that bridging assays are less sensitive or more difficult to develop than other methods Proposed change: Delete: "However, this procedure may be less sensitive than other immunoassay methods and can require significant development effort to produce a suitable assay."	Comment partly accepted. The wording was changed from 'can' to 'may'.
199-200	10	Comment: There is no evidence that bridging assays are less sensitive or more difficult to develop than some other methods.	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change: Delete "However, this procedure may be less sensitive than other immunoassay methods and can require significant development effort to produce a suitable assay."	Comment partly accepted. The wording was changed from 'can' to 'may'.
199-200	7	Comment: This sentence could be challenged. Bridging assays can have quite good relative sensitivity but, as with all such assays, this is very dependent on their design and on the affinity of the positive control that is used to gauge assay sensitivity. Solution phase bridging assays have proven to be sensitive and robust, especially when using electrochemiluminescence detection. However, these assays are sensitive to interference from the target of the therapeutic. Proposed change (if any): Remove the sentence regarding bridging assays being less sensitive and requiring significant development work. Add "It should be noted that these assays are prone to false positives from the target of the therapeutic, especially from a protein that circulates. Therefore confirmation of a positive result is recommended."	Comment partly accepted. The guideline already states this.
199-200	11	Comment: This sentence is incorrect. Bridging assays can have quite good relative sensitivity but, as with all such assays, this is very dependent on their design and on the affinity of the positive control that is used to gauge	Comment not accepted

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		assay sensitivity. Proposed change (if any): Please clarify.	We feel like the guideline text is already clear.
200-201	11	Comments: "It will also not efficiently detect IgG4 antibodies..". Please discuss the general relevance of analysis of IgG4 antibodies. This should consider the fact that an IgG4 response is always accompanied or at least preceded by another Ig subtype response. A bridging ELISA may be able to detect IgG4 antibodies since no all IgG4 antibodies are monovalent (swapped). In addition elevated concentrations of drug-specific IgG4 antibodies will be detectable since self-"swapping" will lead to bivalent molecules again detectable by bridging ELISA.	Comment accepted The IgG4 problem is simply mentioned in the guideline. But detection will be less efficient.
201-202	7	Comment: IgG4 does not activate the complement system and in our experience, IgG4 is always accompanied by IgG1, IgG2 or IgG3. Therefore an anti-drug antibody response will be detected and a specific test for IgG4 would not provide additional information. It would be preferable for this to be acknowledged in the guidance to avoid unnecessary testing and for the text of the guidance to be rewritten so that it does not imply that a validated test for IgG4 is a requirement. In addition, there is evidence that the IgG4 response is	Comment not accepted. The guideline does not imply that a validated test for IgG4 is a requirement.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		not relevant for allergic reactions. Often high IgG4 levels do not correspond to clinical symptoms. See Allergy 2008; 63: 793-6 Proposed change (if any): Delete sentence: "It also will not efficiently detect the IgG4 antibodies which can be produced in some cases."	This statement should be kept in the guideline.
202-203	7	Comment: Anti-Immunoglobulin reagents are not needed for either ELISA or SPR screening assays. They may be needed for confirmatory purposes. Proposed change (if any): Delete the sentence "This does not…against mAbs" at this section."	This is what guideline says. Comment not accepted
202-203	11	Comment: Anti-Immunoglobulin reagents are not needed for either ELISA or SPR screening assays. They may be needed for confirmatory purposes. Proposed change (if any): Delete the sentence "This does not…against mAbs" at this section."	Comment not accepted As above
203-204	4	Comment: SPR is not truly real time; although, it is faster than other methodologies. In addition, the relevance of real-time is not clear. Proposed change (if any): **It is ~~a real time procedure and is therefore~~ fast and also detects rapidly dissociating antibodies which can be missed by other methods.**	Comment not accepted SPR is generally regarded as being as close to real time as you can get.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
205-206	7	Comment: While SPR assays are typically low throughput, they can be developed to have relative sensitivities that are in the low ng/ml range for high affinity Abs, which is well within the Mire Sluis et al white paper recommendation for ATA assay sensitivity. Proposed change (if any): Please clarify.	Comment not accepted It was not understood what needed to be clarified.
205-206	11	Comment: While SPR assays are typically low throughput, they can be developed to have relative sensitivities that are in the low ng/ml range for high affinity Abs, which is well within the Mire Sluis et al white paper recommendation for ATA assay sensitivity. Proposed change (if any): Please clarify.	Comment not accepted. It was not understood what needed to be clarified.
205-207	9	Comment: SPR is capable of detecting high affinity antibodies in the low ng/ml range—easily within current white paper guidelines, and may be better at detecting low affinity antibodies, more generally produced against humanized MAbs, than typical ELISA formats. Proposed change: Delete "comment on SPR sensitivity for detecting high affinity antibodies.	Comment not accepted This guideline does not refer to white paper guidelines and it does not necessarily agree with parts of the white papers.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
208-113	6	Comment: What are "acceptable" levels for Background, sensitivity, specificity and how should they be proven? Proposed change (if any): Please specify, which acceptance criteria should be applied and the consequences on failure.	Comment not accepted This is decided on a case-by-case basis. Further specific information cannot be provided.
208-211	4	Comment: In addition, the product itself may interfere with the anti-mAb antibody detection. Although the next paragraph (7.2) covers this aspect, this should also be mentioned here. Proposed change (if any): "Samples (normally serum or plasma) may contain substances that interfere with the assays **i.e. matrix effect** which produce false positive or negative results and/or incorrect assessment of antibody content. Well known examples of this are complement components, mannose binding protein, Fc receptors, complement receptor 1 and rheumatoid factors, but other substances **even the product itself (see section 7.2 for details)** can also cause problems**".**	Comment accepted.
208-213	7	Comment: "Samples (normally serum or plasma) may contain substances that interfere with the assays which produce false positive or negative results and/or incorrect assessment of antibody content. Well known examples of this are complement components, mannose binding	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		protein, Fc receptors, complement receptor 1 and rheumatoid factors, but other substances can also cause problems. Assays often need to be 'tailored' to reduce artefacts and achieve acceptable background signal levels, sensitivity and specificity." In addition to the examples listed that could potentially interfere with the assays, soluble targets (ligands) deserve a sentence or two because it is a practical concern for all mAbs with soluble targets especially when a bridging immunogenicity assay is used. In this case, the soluble targets or ligands block both the capture and detection reagent(s) from binding to the antibodies. Interference with soluble targets or ligands should be built in the assay validation package as appropriate. Proposed change (if any): It is recommended that a section is devoted to a discussion on the interactions among mAb, antibodies, target, and their respective assays. For example, depending on the PK assay format (total vs. free, and epitopes involved), presence of soluble target and antibodies may or may not appear to impact mAb PK. Soluble target/ ligand and mAb may interfere with the bridging immunogenicity assay. And presence of mAb and antibodies in the sample could also induce high variability in total soluble target/ligand results. This is relevant because the interactions may complicate the result interpretation. Adequate testing and	This is now included in the text. A discussion on PK issues is beyond the remit of this guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		documentation of the assay performance is critical for data	
208-213	11	Comment: "Samples (normally serum or plasma) may contain substances that interfere with the assays which produce false positive or negative results and/or incorrect assessment of antibody content. Well known examples of this are complement components, mannose binding protein, Fc receptors, complement receptor 1 and rheumatoid factors, but other substances can also cause problems. Assays often need to be 'tailored' to reduce artefacts and achieve acceptable background signal levels, sensitivity and specificity." In addition to the examples listed that could potentially interfere with the assays, soluble targets (ligands) deserve a sentence or two because it is a practical concern for all mAbs with soluble targets especially when a bridging immunogenicity assay is used. In this case, the soluble targets or ligands block both the capture and detection reagent(s) from binding to the antibodies. Interference with soluble targets or ligands should be built in the assay validation package as appropriate. Proposed change (if any): It is recommended that a section is devoted to discuss the interactions among mAb, antibodies, target, and their respective assays. For example, depending on the PK assay format (total vs. free, and epitopes involved), presence of soluble target and antibodies may or may	Comment partly accepted. A discussion on PK issues is beyond the remit of this guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		not appear to impact mAb PK. Soluble target/ ligand and mAb may interfere with the bridging immunogenicity assay. And presence of mAb and antibodies in the sample could also induce high variability in total soluble target/ligand results. This is relevant because the interactions may complicate the result interpretation. Adequate testing and documentation of the assay performance is critical for data interpretation, such as whether antibodies are clearing and/or neutralizing in nature.	
209-211	7	Comment: "Well known examples of this are complement components, mannose binding protein, Fc receptors, complement receptor 1 and rheumatoid factors….." We are not sure how well known this particular set of potential ATA assay interferants is. It would be helpful to include evidence or literature reports of ATA interference by C', MBPs, FcRs, or CR1. Proposed change (if any): Please add literature citations if these are available.	Comment not accepted References are not included in regulatory guidelines.
209-211	11	Comment: "Well known examples of this are complement components, mannose binding protein, Fc receptors, complement receptor 1 and rheumatoid factors….." We are not sure how well known this particular set of potential ATA assay interferants is. It would be helpful to include evidence or reports of ATA interference by C',	Comment not accepted References are not included in regulatory guidelines.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		MBPs, FcRs, or CR1. Proposed change (if any): Please add literature citations if these are available.	
210	7	Comment: Interference from therapeutic target proteins should be tested. Proposed change (if any): Add "therapeutic target" to list of possible interferents.	Comment accepted.
214	7	Comment: The header wording may raise misunderstandings. Proposed change (if any): Change to "Drug interference, relevance and strategies to overcome"	Comment partly accepted. Wording was changed in the guideline text.
214	11	Comment: The header wording may raise misunderstandings. Proposed change: Change to "Drug interference, relevance and strategies to overcome"	Comment partly accepted. Wording was changed in the guideline text.
214-233	10	Comment: Presence of a mAb product in sample may complicate the analysis as described here. Emerging science around modelling of immune responses and new assays may overcome these problems, e.g. B cell	Comment not accepted

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		ELISpot for determining presence of B cells producing an ADA. Furthermore, modelling of immune response together with the PK of the mAb could allow determination of the optimal time for sampling to determine the presence of an ADA. Alternative methods may be required to measure the presence of an ADA, e.g. by B cell ELISpot.	A discussion on PK is beyond the remit of this guideline. B cell ELISpot is not usually used for this.
215	9	Comment: "High dose" is a relative term in the absence of a statement of what it constitutes. Moreover, even when a mAb is administered at low doses, the presence of the product in samples collected for antibody assessment can interfere with accurate assessments. We recommend removal of the first sentence on line 215. Proposed change: The statement that "mAb products are usually administered in relatively high doses" should be removed.	Comment accepted.
215	2	Comment: The discussion of the presence of mAb product in samples begins with the observation that mAb products "are usually administered in relatively high doses." The nature of the comparison (i.e., relative to what) is not apparent. Moreover, even when a mAb is administered at low doses, the presence of the product in samples	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		collected for antibody assessment can interfere with accurate assessments. We recommend that the CHMP delete the first sentence on line 215. Proposed change (if any): "~~MAb products are usually administered in relatively high doses.~~"	
215	7	Comment: The first sentence in section 7.2 is somewhat ambiguous because it does not explain the basis of the comparison (i.e. relatively high doses compared to what products). The sentence is also unnecessary because even when mAbs are administered at low doses, the presence of the product in collected samples can affect estimates of antibody content. Accordingly, the sentence should be deleted. Proposed change (if any): The sentence ´Mab products are usually administered in relatively high doses.' should be deleted	Comment accepted.
215	8	**Comment:** The discussion of the presence of mAb product in samples begins with the observation that mAb products "are usually administered in relatively high doses." The nature of the comparison (i.e., relative to what) is not apparent. Moreover, when a mAb is administered at low doses, the presence of the product in samples collected for antibody assessment can interfere with accurate assessments. **Proposed change**: "~~MAb products are usually~~	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		~~administered in relatively high doses.~~ **MAbs** have […]".	
215-233	6	Comment: • This discussion is too detailed. Recommendations regarding different assay types and technologies should not be part of a guideline since methods can change and / or specific vendors of technologies can profit. • We do not know the effects of acid dissociation on ADA and this must not be overlooked. Acid dissociation can be considered in cases where it is needed to detect ADA in samples containing high levels of drug when adverse effects have been observed. End of study analysis where drug levels are lower, should be measured without an acid dissociation step. • In general this paragraph is very speculative and appears to be more a scientific publication text than guidance. Speculations do not give the correct guidance (e.g. no examples are given for when bridging assay wouldn't suffice or when a washout period in the dosing schedule would not be the right approach). Proposed change (if any): Please remove this section.	Comment not accepted. We do not agree. This is a major technical problem with measuring antibodies against therapeutic antibodies and must be considered in the guideline.
223-224	5	Comment: It is said that the ADA response declines as fast as the	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		drug is eliminated. However, the drug can induce an immune response as long as it is present. This means that it should be possible to identify a sampling time point after the drug is cleared before the ADA response is gone. In our opinion the only problem with wash-out samples is that they will fail to detect transient immune responses. Proposed change (if any): Please revise accordingly	This is what is said in the guideline.
223-224	7	Comment: It is said that the ADA response declines as fast as the drug is eliminated. But, the drug can stimulate the immune response as long as it is present. This means that it could be possible to identify a sampling time point after the drug is cleared before the ADA response starts to decline Proposed change (if any): Please revise accordingly	Comment not accepted. This is what is said in the guideline.
224	10	Comments: 'ECL' is not defined Proposed change: Define above abbreviation.	Comment accepted.
224-226	7	Comment: It is possible to very carefully design bridging ATA assays to minimize mAb interference. ATA assay sensitivity is a complex area, which is driven by a number of variables. It would be useful to refer to	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		recent publications in this area. The statement that "some ECL based immunoassays seem much less affected by residual product in samples…" is vague and does not provide useful guidance. Provided the statement on ECL is included, please discuss this in the context of one-step bridging ELISAs which have been shown to have comparable drug tolerance to ECL assays. Proposed change (if any): Please consider referring to existing literature in this area. Consider deletion of the statement on advantages of ECL.	It is considered that this is true and clear as stated. References are not included in regulatory guidelines.
224-226	11	Comment: It is possible to very carefully design bridging ATA assays to minimize mAb interference. ATA assay sensitivity is a complex area, which is driven by a number of variables. It would be useful to refer to recent publications in this area. The statement that "some ECL based immunoassays seem much less affected by residual product in samples…" is vague and does not provide useful guidance. Provided the statement on ECL is included, please discuss this in the context of one-step bridging ELISAs	Comment not accepted. It is considered that this is true and clear as stated.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		which have been shown to have comparable drug tolerance to ECL assays. Proposed change (if any): Please consider referring to existing literature in this area. Consider deletion of the statement on advantages of ECL.	References are not included in regulatory guidelines.
227-229	9	Comment: Use of various biochemical disruption agents to dissociate Ab-drug complexes can have unknown deleterious effects on patient samples potentially containing antitherapeutic antibodies, even when a method has been optimized using a surrogate positive control. Published data has demonstrated that some Abs are sensitive to acid dissociation, for instance. Other, less harsh methods may be preferred. Proposed change: Remove recommendation of acid dissociation as a method to disrupt Ag-drug complexes.	Comment not accepted. We do not agree with this comment. This is a major technical problem with measuring antibodies against therapeutic antibodies and it must be considered in the guideline.
227-228	7	Comment: Biochemical disruption approaches can be used to try to minimize mAb interference. However, such methods have been shown to negatively impact some Ag/Ab interactions upon subsequent testing. Though optimization of such procedures with a positive control ATA can show better recovery of that control in the	Comment not accepted. We do not agree with this comment. This is a major technical problem with measuring antibodies against therapeutic antibodies and it must be considered in the guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		presence of the therapeutic mAb, the impact of these approaches on an unknown polyclonal Ab ATA sample cannot reasonably be anticipated or accounted for. Proposed change (if any): Please recognize the caveats of using disruptive biochemistries in this context.	
227-228	11	Comment: Biochemical disruption approaches can be used to try to minimize mAb interference. However, such methods have been shown to negatively impact some Ag/Ab interactions upon subsequent testing. Though optimization of such procedures with a positive control ATA can show better recovery of that control in the presence of the therapeutic mAb, the impact of these approaches on an unknown polyclonal Ab ATA sample cannot reasonably be anticipated or accounted for. Proposed change (if any): Please recognize the caveats of using disruptive biochemistries in this context.	Comment not accepted. We do not agree with this comment. This is a major technical problem with measuring antibodies against therapeutic antibodies and it must be considered in the guideline. Already in guideline.
228-231	11	Comment: There is a risk of generating false-negative results by acid dissociation due to denaturation of acid-labile ADAs. We propose to only perform acid dissociation if sample is screening negative, drug levels are above the tolerance level of the assay and other factors / events point to the existence of ADAs (such as decline in PK). Proposed change: Add the point above	Comment not accepted. This proposal is too detailed. Discussion of PK issues is beyond the remit of this guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
229-231	3	Comment: Experimental data indicate that it is the ratio of the concentration of drug product relative to the level (avidity and concentration) of ADA that is the determining factor for drug tolerance threshold. Thus, it s questionable whether dilution of the sample will alleviate drug interference. Proposed change (if any): Please delete "*A final possibility is to dilute samples so that residual product present is insufficient to interfere with the assay*."	Comment not accepted. We do not agree with this comment. We do not agree with this comment. This is sometimes done.
233	4	Comment On Line 233, assay validation typically considers some combination of dilution, acid disassociation, and delay in sampling. Proposed change (if any): Add the following sentence at end of line 233: ***"In many cases, anti-drug antibody method development, validation, and testing utilizes a combination of all three approaches to reduce drug interference"***	Comment accepted.
233	7	Comment: It is unclear which samples need to be assayed for residual drug. It is assumed that the assay has been fully validated to detect antibodies in the presence of	Comment not accepted. This proposal is too detailed for a guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		drug. Proposed change (if any): Clarify whether the residual drug needs to be determined in diluted samples or undiluted samples and the reasoning behind it.	
234-238	3	Comment: An increasing number of mAb-related products are manufactured using microbial cell substrates. This raises the possibility that pre-existing and/or treatment-emergent antibodies reactive with host cell-derived factors might be detected in the ADA assay. Proposed change (if any): In the case of production of a mAb-related product using microbial host cell substrate, the confirmatory assay might need to include competing antigens that represent potential process-related impurities.	Comment not accepted This proposal is too detailed for a guideline. A part of this proposal is already covered in the general guideline.
234-238	7	Comment: The paragraph describes an important aspect, however, it does not discuss specific considerations for mAb development. Proposed change (if any): Consider to rename the section as "Confirmatory and characterization assays" and add lines 247-249 to that section and provide guidance under what circumstances	Comment not accepted The comment is not entirely understood. The assay description is not 'characterization'. The quoted lines refer to controls and

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		this characterization may be necessary. Chapter 8 (lines 250-273) could, consequently, be a subchapter of this.	are not relevant here.
234-238	11	Comment: The paragraph describes an important aspect, however, it does not discuss specific considerations for mAb development. Proposed change: Consider to rename the section as "Confirmatory and characterization assays" and add lines 247-249 to that section and provide guidance under what circumstances this characterization may be necessary. Chapter 8 (lines 250-273) could, consequently, be a subchapter of this.	Comment not accepted The comment is not entirely understood. The assay description is not 'characterization'. The quoted lines refer to controls and are not relevant here.
236-238	7	Comment: We suggest using the term "competitive inhibition" to improve the overall clarity of this section. Proposed change (if any): The most common approach for this is ~~to include an incubation step with the mAb product in the assay to show that this results in a significantly diminished signal when assaying real antibody positive samples~~ the addition of a competitive inhibition step in the screening assay. Significantly diminished signal resulting from an incubation step with the mAb product confirms that the assay is measuring drug-specific antibody."	Comment not accepted The proposed change is not as clear as the original text.
236-238	8	**Comment:** We suggest using the term "competitive inhibition" to improve the overall clarity of this section.	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change: The most common approach for this is ~~to include an incubation step with the mAb product in the assay to show that this results in a significantly diminished signal when assaying real antibody positive samples~~ **the addition of a competitive inhibition step in the screening assay. Significantly diminished signal resulting from an incubation step with the mAb product confirms that the assay is measuring drug-specific antibody**."	The proposed change is not as clear as the original text.
239-249	10	Comment: Discussion around positive control suggests that the best choice is to use human serum with positive anti-drug reactivity and that the use of non-human positive control is acceptable but is not most favourable. It is also suggested that assay sensitivity and specificity is driven by the positive control serum chosen for the assay. These suggestions require clarification since the positive control by itself does not represent a real study sample. Use of non-human anti-drug antibodies as positive control in immunogenicity assays has been widely viewed as acceptable. It is also suggested that anti-idiotype anti-serum can in some cases provide a useful positive control – a statement that requires clarification. Proposed change: Explain that the use of a non-human anti-drug antibody is acceptable as positive control in the assay. It has to be verified that the assay is able to detect various types of anti-drug activities, including	Comment not accepted. The proposed version does not differ from the original.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		anti-CDR and anti-Fc.	
240-249	5	Comment: a) Even in late stages of product development it might not be feasible either due to ethical reasons or due to the amount of available serum to use human sera as positive control. b) Animal welfare in several European countries (if not in all) does currently not allow immunizing non-human primates just to produce positive controls. c) Serum from hyperimmunized animals can be depleted from anti-Fc antibodies (using a human isotype to the mAb). This might be an alternative to monoclonal anti-idiotype antibodies. d) For immunisation of non-primate species the $F(ab')_2$ fragment of the mAb might be used to exclude the production of antibodies against the Fc part of the MAb. Proposed change (if any): Please revise accordingly	Comment not accepted. This proposal is too detailed for a guideline.
240-249	6	Comment: • The last sentence "For confirmatory assays, spiking samples with an irrelevant mAb or (better) with a mAb with the same Fc but different CDRs as the product can be used to confirm specificity" doesn't make sense: Antibodies against the Fc part can be highly relevant as stated further down in the	Comment partly accepted. This suggestion was implemented.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		guideline. • Regarding the statement "The chosen positive control serum affects sensitivity and specificity of the immunogenicity assay" it should be mentioned that any type of monoclonal or recombinant anti-idiotypic antibodies are relevant as positive controls. The positive control does not affect sensitivity and specificity of the assay; it affects the results of the sensitivity and specificity measurements. Please rephrase. • If the use of animal derived ADA as positive control is acceptable then please describe in this paragraph that this is sufficient. Proposed change (if any): • The following part should be deleted: "If human sera are not available (as is likely during early phases of product development) then the use of animal sera is the only option. Choice of species for this has important consequences. Non-human primates produce primarily anti-CDR responses against human or humanized mAbs, which may closely mimic human responses. However, non-primate species usually produce antibodies primarily against the constant regions of the mAb, which is unlike human responses. Use of an anti-idiotypic antiserum or mAb can, in some	This suggestion was implemented. This is decided on a case-by-case basis. This is not correct and cannot be accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		cases, provide a useful positive control." And replaced with: "Any type of monoclonal or polyclonal anti-idiotypic antibodies are relevant as positive controls. For pre-clinical assays also non-anti-idiotypic antibodies can be used as well." The last sentence should be deleted.	
241	9	Comment: The sensitivity and specificity of the assay is intrinsic to the assay format and is not affected by the chosen positive control. Proposed change: Replace:"The chosen positive control serum affects sensitivity and specificity of the immunogenicity assay." with: "The positive control choice can be important in monitoring assay sensitivity and selectivity."	Comment accepted.
241	10	Comment: The sensitivity and specificity of the assay is intrinsic to the assay format and is not affected by the chosen positive control. Proposed change: The positive control choice can be important in monitoring assay sensitivity and selectivity. Please clarify in the final guidance.	Comment accepted.
241	4	Comment: Purified antibodies can also be used as positive control Proposed change (if any): *The chosen positive control* ***e.g.*** *serum* ***or purified***	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		antibodies *affects sensitivity and specificity of the immunogenecity assay."*	
241	7	Comment: The positive control is only a surrogate to analyze for sensitivity. Proposed change (if any): Please rewrite the sentence to "…affects apparent sensitivity and…"	Comment not accepted. This proposal is too detailed for a guideline.
241	11	Comment: The positive control is only a surrogate to analyze for sensitivity. Proposed change: Please rewrite the sentence to "…affects apparent sensitivity and…"	Comment not accepted. This proposal is too detailed for a guideline.
242	4	Comment: Also in late stages of product development it might not be feasible (due to ethical reasons, as in many cases cancer patients are the treatment population, or due to the amount of available serum) to use human sera as positive control. Proposed change (if any): *"If human sera are not available (as is likely during ~~early phases of~~ product development) then use of animal sera is the only option."*	Comment accepted. Text was modified.
242-245	7	Comment: In our experience, human sera are never available in sufficient amounts to be used as positive controls for ATA assays. If the purpose of a positive control is to show that the analytical method is working (or not)	Comment accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		then controls from sources other than humans (non human primates, rodents) should be acceptable for bridging assays. Proposed change (if any): Please clarify.	Text was modified.
242-245	11	Comment: In our experience, human sera are never available in sufficient amounts to be used as positive controls for ATA assays. If the purpose of a positive control is to show that the analytical method is working (or not) then controls from sources other than humans (non human primates, rodents) should be acceptable for bridging assays. Proposed change (if any): Please clarify.	Comment accepted. Text was modified.
242-247	7	Comment: This statement should be reworded as it indicates the only other option for a positive control when human serum is not available is non human primate serum, but then it goes on to say that monoclonal antibodies are OK… Proposed change (if any): Please re-word.	Comment not accepted. The guideline does not state this.
242-247	11	Comment: This statement should be reworded as it indicates the only other option for a positive control when human	Comment not accepted. The guideline does not state this.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		serum is not available is non human primate serum, but then it goes on to say that monoclonal antibodies are OK… Proposed change (if any): Please re-word.	
243-247	7	Comment: Each human ATA response will be unique, so trying to "mimic" a human response is not practical. Since positive controls are used to help develop and control an ATA assay, even a murine anti-idiotype antibody can be a satisfactory positive control. Monoclonal antibodies also have the advantage of being consistent reagents, which polyclonal sera are not. Anti-CDR specific antibodies should be a positive control for all mAb therapeutics independent from which species they have been acquired. A pAb preparation may have to the advantage of being a mixture of different affinities and epitopes: it is, however, still not a replication of human response. Proposed change (if any): Please clarify and rephrase. The human immune response to protein therapeutics is individually and temporally unique and positive controls from any species cannot be expected to mimic these responses. Include that pAb could be superior to a mAb	Comment not accepted. This comment is not true.
243-247	11	Comment: Each human ATA response will be unique, so trying to	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		"mimic" a human response is not practical. Since positive controls are used to help develop and control an ATA assay, even a murine anti-idiotype antibody can be a satisfactory positive control. Monoclonal antibodies also have the advantage of being consistent reagents, which polyclonal sera are not. Anti-CDR specific antibodies should be a positive control for all mAb therapeutics independent from which species they have been acquired. A pAb preparation may have to the advantage of being a mixture of different affinities and epitopes: it is, however, still not a replication of human response. Proposed change (if any): Please clarify and rephrase. The human immune response to protein therapeutics is individually and temporally unique and positive controls from any species cannot be expected to mimic these responses. Include that pAb could be superior to a mAb	This comment is not true.
243-245	7	Comment: "Non-human primate produce primarily anti-CDR responses [...]". Our experience is that non-human primates produce both anti-CDR and anti-framework antibodies. Proposed change (if any): "Non-human primates produce ~~primarily~~ both anti-CDR ~~responses against human or humanized mAbs~~ and anti-Ig framework antibodies "	Comment accepted. Text has been modified.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
243-245	8	**Comment: "**Non-human primates produce primarily anti-CDR responses [...]". Our experience is that non-human primates produce both anti-CDR and anti-framework antibodies. However, whether the non-human primate response most closely mimics the potential human response is a topic that is frequently challenged. **Proposed change: "**Non-human primates produce ~~primarily~~ **both** anti-CDR ~~responses against human or humanized mAbs~~ **and anti-Ig framework antibodies**, ~~which~~ **and** may ~~most closely mimic~~ **be an appropriate control.** ~~human responses~~".	Comment accepted. Text has been modified.
244-247	9	Comment: "Choice of positive control source has importantconsequences" statements misconstrue the rather restrictive role that positive controls play in immunogenicity assays. Anti-CDR or anti-ID Abs from any animal source will equally suffice for controlling these assays. In general, screening assays are not formatted to distinguish anti-CDR from antiframework, so the distinction is irrelevant. Proposed change: Remove recommendation that primate derived positive controls are preferred.	Comment not accepted. This is not a recommendation, so it cannot be removed.
245-246	4	Comment: For immunisation of non-primate species the $F(ab')_2$ or Fab' fragment of the mAb may be used to minimise the	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		risk of producing antibodies against the Fc part of the mAb. Proposed change (if any): "However, **immunisation of** non-primate species ~~usually produce antibodies primarily against the constant regions of the mAb, which is unlike human responses~~ **using the F(ab')$_2$ or Fab' fragment of the mAb might be an option to obtain anti-CDR antibodies which can mimic the human responses and, thus, can serve as a relevant positive control."**	The proposed wording is equivalent to the existing one.
246-247	4	Comment: Use of Anti-Fab may also be of benefit as a positive control. Proposed change (if any): *Use of an anti-idiotypic antiserum, **anti-Fab**, or mAb can, in some cases, provide a useful positive control."*	Comment not accepted. The proposal is too detailed for a guideline.
247	7	Comment: However, it is unclear why anti-idiotypic antisera for mAb therapeutics are only in "some cases" a useful positive control. Proposed change (if any): Delete "in some cases" from the sentence.	Comment not accepted. The 'Some cases' wording is necessary, as the antisera are not always useful.
247	11	Comment: However, it is unclear why anti-idiotypic antisera for mAb therapeutics are only in "some cases" a useful	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		positive control. Proposed change: Delete "in some cases" from the sentence.	The 'Some cases' wording is necessary, as the antisera are not always useful.
247-249	10	Comment: There is only one sentence touching on the importance of the negative control. Proposed change: Selection of the assay negative control should be further explained. Please clarify that negative control is expected to behave similar to the study population matrix without specific anti-drug reactivity.	Comment not accepted. This is rather obvious and is included in the general guideline.
248-249	9	Comment: The guideline discusses the use of isotype-matched non-reactive antibodies in confirmatory assays to confirm/demonstrate specificity of the ADA detection method. For a validated method whose specificity was adequately demonstrated, this type of specificity control is highly unnecessary. Proposed change: It should be indicated that the use of isotype-matched non-reactive antibodies in confirmatory assays should occur during method development and is not a requirement during study phase bioanalysis.	Comment not accepted. This proposal is too detailed for a guideline.
248-249	8	**Comment:** The statement, "For confirmatory assays, spiking samples with an irrelevant mAb or (better) with a mAb with the same Fc but different CDRs as the product can be used to confirm specificity," would be	Comment partly accepted. Text was modified, but was not moved as it would be incorrect to change section.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		more appropriately placed in the Section 7.2 "Confirmatory assays" and modified for clarity. **Proposed change:** We suggest moving to Section 7.2 and re-wording as follows: "For confirmatory assays, spiking samples with an irrelevant mAb, or ~~(better)~~ with a mAb with the same Fc but different CDRs as the product, can be used to ~~confirm~~ **additionally characterize the** specificity **of the immunogenicity response**."	
248-249	2	Comment: The use of isotype-matched non-reactive antibodies is suggested to confirm specificity. This is important for demonstrating the specificity of the test method during method validation, but it adds no value during study phase bioanalysis. Proposed change (if any): We recommend that the text be clarified to indicate that the use of isotype-matched non-reactive antibodies for demonstrating specificity is expected during method validation.	Comment not accepted. Existing text is already clear.
248-249	7	Comment: The statement "For confirmatory assays, spiking samples with an irrelevant mAb or (better) with a mAb with the same Fc but different CDRs as the product can be used to confirm specificity" would be more appropriately placed in the Section 7.2/7.3 and	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		modified for clarity or preferably deleted. Proposed change (if any): We suggest either deletion or moving to Section 7.2/ 7.3. and re-wording as follows: "For confirmatory assays, spiking samples with an irrelevant mAb, or ~~(better)~~ with a mAb with the same Fc but different CDRs as the product, can be used to ~~confirm~~ additionally characterize the specificity of the ~~immunogenicity~~ anti drug antibody response."	Text was modified, but was not moved as it would be incorrect to change section.
248-249	10	Comment: "To confirm specificity" needs to be clarified since antibodies could bind to shared epitopes on the irrelevant mAb Proposed change: "to *confirm identify* specificity *for a particular region (e.g. CDR) of the mAb*.	Comment accepted. Text was modified.
249	9	Comment: "to confirm specificity" needs to be clarified since anti-drug antibodies could bind to shared epitopes on the irrelevant mAb Proposed change: Modify current text to state: ... to identify specificity for a particular region (e.g. CDR) of the mAb	Comment accepted. Text was modified.
249	4	Comment: Spiking with an irrelevant mAb with the same Fc as the product may not show specificity for the confirmation assay in a nonclinical setting owing to the anti-human Fc response in animals.	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Adding a clarifying sentence on Line 249: ***"Spiking with an irrelevant mAb with the same Fc as the product may not show specificity for the confirmation assay in a nonclinical setting owing to the anti-human Fc response in animals".***	Text was modified. Guideline does not specifically relate to non-clinical settings.
249	7	Comment: The draft guideline recommends that mAb applicants use isotype matched non-reactive antibodies to measure specificity of the assay. The guideline is somewhat unclear, however, on when this specificity testing should be conducted. These antibody controls are critical to assay method validation, but they are not useful once the applicant reaches the bioanalysis study phase. The CHMP should therefore clarify that the specificity testing should be completed during assay method validation. Proposed change (if any): The following sentence should be added at the end of line 249: "The specificity of the assay should be determined during the assay method validation process. Specificity of ATA should be verified for each sample that is reactive in a screening assay. This is frequently accomplished through competitive inhibition in a confirmatory assay."	Comment not accepted. Guideline does not recommend this. It just states that such an approach may be useful. This in sect. 7.3. Not applicable to line 249.
252-260	5	Comment:	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		A dedicated NAb assay might not be required in the preclinical setting (as immunogenicity in preclinical setting is not predictive for humans). Proposed change (if any): An integrated assessment of pharmacokinetics, pharmacodynamics and immunogenicity of the drug can aid in an interpretation of whether the in vivo activity of the drug is being adversely affected either by direct neutralization or by increased clearance. A respective sentence should be added.	This section of the guideline is not specifically related to non-clinical settings. The above does not affect the need for a NAB assay.
252-260	6	Comment: Major compliment for stating that binding assays are usually sufficient for mAb neutralization tests! This seems a breakthrough in this discussion. Proposed change (if any): • Please mention explicitly that NAb assays are not required for pre-clinical studies (Preclinical ADA findings are not directly translatable to clinic) • An integrated assessment of pharmacokinetics, pharmacodynamics and immunogenicity of the drug can aid in an interpretation of whether the in vivo activity of the drug is being adversely affected either by direct neutralization or by increased clearance. A respective sentence should be added.	Comment not accepted. This section of the guideline is not specifically related to non-clinical settings. The above does not affect the need for a NAB assay.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
252-273	4	Comment: According to the available literature in case of a mAb directed against a soluble target, the "competitive ligand binding assay" is the choice for neutralizing capacity testing. However, for mAbs directed against a cell surface bound targets, "cell based assays" are more applicable. In the first paragraph (lines 252-260) the text says that for mAbs in general the "competitive ligand binding assay" is the most suitable method, however the 2nd paragraph (lines 261-273) says, not in all cases. Proposed change (if any): Recommend adding the following sentence in line 270: *"… considered to be the primary mode of action.* ***In this regard, a cell based potency approach has an advantage."***	Comment accepted.
253-254	9	Comment: "It is normally expected that [Nab activity] be measured." In this regard the guidance is not clear at what stage of development this needs to occur and whether the risk-based approach is the basis for such decisions. As opposed to replacement factors or cytokine biotherapeutics, the consequences of Nabs against mAbs is basically loss of biological activity, which can be assessed by loss of efficacy. Other safety issues are no different than non-Nabs (i.e., binding Abs)—see comment line 361-364, below.	Comment partly accepted. Risk covered in risk section.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change: This recommendation/assumption should be informed by the risk assessment for molecules, which for mAbs as a class is generally low or very occasionally moderate.	
253-254	8	**Comment:** "It is normally expected that the neutralizing capacity of any antibodies induced is measured." The term "measured" suggests that a neutralizing antibody assay must be performed. However, there may be situations where a neutralizing assay is not feasible, and an alternative method for assessing neutralization of the drug is considered. **Proposed change:** "It is normally expected that the neutralizing capacity of any antibodies induced is measured. **However, in the event of a demonstrated inability to develop a neutralizing antibody assay, consideration of alternative methods for assessment of neutralizing activity (e.g. pharmacodynamic marker measurement) should be discussed with regulatory authorities."**	Comment not accepted. We are not aware of such examples. Nab assay is deemed necessary. PD measures differ from Nab assays. PD aspects are not covered in this guideline.
253-254	7	Comment: Please clarify that the need to assess the neutralizing capacity of an ATA response is also tied to the risk-based approach described in section 9. Proposed change (if any): Characterization of ATAs in a Nab assay should be risk-based and scientifically	Comment not accepted. Both assays are required.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		driven. Comment/ Proposed change (if any): Please discuss the relevance of Nabs compared to the relevance of binding antibodies in that section again since binding antibodies might be more relevant than neutralizing Abs (see line 339 and comments thereto) Comment: "It is normally expected that the neutralizing capacity of any antibodies induced is measured." There may be situations where a neutralising antibody assay is not feasible and alternate methods for assessing neutralisation of the drug should be considered. Proposed change (if any): "It is normally expected that the neutralizing capacity of any antibodies induced is assessed. However, under some circumstances alternative methods for assessment of neutralising activity (e.g. pharmacodynamic marker measurement) may be justified, and discussed with regulatory authorities.	
253-254	11	Comment: Please clarify that the "normal" need to assess the neutralizing capacity of an ATA response is tied to the risk-based approach described in section 9.	Comment not accepted. Please see above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Characterization of ATAs in a Nab assay should be risk-based and scientifically driven, not automatic. Comments/ proposed change: Please outline why "it is normally expected that the neutralizing capacity of any antibodies induced is measured" and discuss the relevance and the availability of PD markers (which are a better predictor of any potential neutralizing activity in vivo) or any other clinical observation to monitor efficacy and the analysis of target-binding competent drug as PK assay and how the strategy is influenced by the risk assessment. We would propose that monitoring a relevant PD marker may provide a more suitable information than a Nab assay when analyzing the in vivo potency of a mAb therapeutic. Please specify what justifies a "deviation". Comments/ proposed change: Please discuss the relevance of Nabs compared to the relevance of binding antibodies in that section again since binding antibodies might be more relevant than neutralizing Abs (see line 339 and comments thereto)	
258-259	7	Comment: Competitive ligand binding assays are not always appropriate for determining neutralizing activity of antibodies.	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Remove sentences and add "Competitive ligand binding assays are useful for assessing the neutralizing potential of detected antibodies if the MAb binds and blocks the ligand as its primary mechanism of action. However, these assays may have limitations or may be difficult to design to reflect the situation in vivo if the MAb is (a) directed towards a target receptor and interferes with the ability of the ligand to bind to it or (b) allows the ligand to bind but interferes with the ability of the receptor to participate in other downstream signalling events, e.g. dimerization, etc, or (c) if the receptor has multiple subunits and the MAb targets only one of the subunits. In the case of (a), if a competitive ligand binding assay is possible to design, availability of the soluble form of the receptor poses a challenge. Also it is challenging to show that the soluble receptor maintains the same conformation as the in vivo form and is biologically active. For all of these reasons, the NAb assay format should be carefully selected and cell-based NAb assays should be considered if the MAb's mechanism of action is more complex than simply blocking or neutralizing ligand."	The proposed wording is deemed as too detailed and not appropriate for a guideline.
261-273	5	Comment: In cases where relevant PD markers are available, these might be more informative for neutralizing capacity of the mAb than in-vitro assays.	Comment not accepted. PD is not equivalent to neutralization as measured with a Nab assay.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Please insert sentence acknowledging this.	
261-273	7	Comment: In the paragraph starting on line 261, the guideline points out that the exact clinical mechanisms of efficacy of a mAb may not be dissectible experimentally, and thus it should not be assumed that "Fc mediated immunobiological effects of the mAbs are not involved in clinical efficacy" for mAbs. If so, it is confusing why "competitive ligand binding assays are often the neutralizing assays of choice for mAbs" (lines 258-259) Proposed change (if any): The selection of a competitive ligand binding assay design for a Nab assay should be justified and the results interpreted carefully. Comment: In cases where relevant, and sensitive PD markers exist, these can also be informative. Proposed change (if any): Please insert sentence acknowledging this.	Comment partly accepted. The text was modified. PD markers are not addressed in this guideline.
261-273	11	Comment: In the paragraph starting on line 261, the guideline points out that the exact clinical mechanisms of efficacy of a mAb may not be dissectible experimentally, and thus it should not be assumed that "Fc mediated	Comment partly accepted. Please see above .

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		immunobiological effects of the mAbs are not involved in clinical efficacy" for mAbs. If so, it is confusing why "competitive ligand binding assays are often the neutralizing assays of choice for mAbs" (lines 258-259) Proposed change (if any): The selection of a competitive ligand binding assay design for a Nab assay should be justified and the results interpreted carefully.	
267-273	5	Comment: The aim of this paragraph is not clear. Is the intention that several neutralizing assays are needed for mAbs having also an effector function (e.g. ADCC)? Proposed change (if any): Text should be rephrased	Comment not accepted. The guideline correctly states that multiple functions of the mAb must be thoroughly characterized.
268-273	8	**Comment:** "[...] care must be taken not to assume [...] Fc [...] not involved [...] In such cases [...] thorough [...] characterization [...] appropriate neutralizing assay strategy." These statements do not connect properly. **Proposed change:** We suggest replacing lines 268-273 with: "**<u>It is important to understand the biologic function of the molecule and to assess neutralizing antibodies appropriately</u>**."	Comment not accepted. We consider that the initial wording should be kept.
268-273	7	Comment: The demonstration of the mode of clinical efficacy of a	Comment not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		mAb as "simple antigen binding" is usually done by "thorough biological characterization of the mAb." The paper seems to suggest that in-spite of this characterization "Fc-mediated immunobiological effects" are assumed. Proposed change (if any): Rephrase lines 268-270 as follows: "Therefore, if intact mAbs are used and unless single antigen binding is demonstrated as the primary mode of action it is to be assumed that Fc-mediated immunobiological effects may be involved in clinical efficacy" Comment: "[...] care must be taken not to assume [...] Fc [...] not involved [...] In such cases [...] thorough [...] characterization [...] appropriate neutralizing assay strategy". These statements do not connect properly. Proposed change (if any): We suggest replacing lines 268-273 with: "It is important to understand the biologic function of the molecule and to assess neutralization appropriately."	This is essentially what the guideline says.
268-273	11	Comment: The demonstration of the mode of clinical efficacy of a mAb as "simple antigen binding" is usually done by "thorough biological characterization of the mAb." The paper seems to suggest that in-spite of this	Comment not accepted. Exactly the same as above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		characterization "Fc-mediated immunobiological effects" are assumed. Proposed change: Rephrase lines 268-270 as follows: "Therefore, if intact mAbs are used and unless single antigen binding is demonstrated as the primary mode of action it is to be assumed that Fc-mediated immunobiological effects may be involved in clinical efficacy"	
274	11	Comment: Risk-based Approach (see General Comment): It should be noted that not all humanized/human mAbs represent "high risk" molecules with regard to immunogenicity. Proposed change (if any): This EMA guidance should to acknowledge the current and widely used industry practise of using risk based assessment of immunogenicity approach for each therapeutic protein and incorporate this into the guideline.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
274-283	3	Comment: This section should be re-located to the front of the guideline, since the risk-based approach represents the basis for the strategy to be applied. Proposed change (if any): Re-locate section to the first part of the guideline. Explain that "risk" refers to the rate of occurrence relative to severity of the clinical consequences of an	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		undesirable immune response to the administered mAb product. The evaluation of the risk should be sufficiently extensive, and should employ methods of appropriate specificity and sensitivity, to detect the pertinent risks. Since the risk factors are likely to be different for different products, or even for different therapeutic indications of the same mAb, it is essential to perform a risk assessment for each particular product and to adapt the strategy for evaluating the level of risk accordingly.	
275	9	Comment: The guideline states that "every therapeutic mAb should be evaluated for immunogenicity". Diagnostic mAbs administered to patients also need to be evaluated and should be added to this statement. Proposed change: Insert "and in vivo diagnostic".	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
275	8	**Comment:** "[...]every therapeutic mAb needs to be evaluated for immunogenicity." We agree, but note that diagnostic mAbs to be administered to patients should be similarly evaluated. **Proposed change:** "Every therapeutic **and in vivo diagnostic** mAb needs to be evaluated for immunogenicity […]."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
275	7	Comment: The draft guideline correctly emphasizes the importance	Comment partly accepted. The clinical and risk sections have been redrafted taking these

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		of studying the immunogenicity of therapeutic mAbs. Immunogenicity, however, is equally relevant to *in vivo* diagnostic mAbs, and companies who develop these products should similarly be required to conduct thorough immunogenicity testing. Proposed change (if any): Every therapeutic and in vivo diagnostic immunogenicity individually" mAb needs to be evaluated for. . .	and other comments into account.
275-276	2	Comment: Section 9 of the draft guideline states that every therapeutic mAb needs to be evaluated for immunogenicity. We agree, but note that diagnostic mAbs to be administered to patients should be similarly evaluated. Proposed change (if any): "Every therapeutic **and in vivo diagnostic** mAb needs to be evaluated for immunogenicity individually and all immunogenicity strategies should be adapted for each mAb development programme."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
275-283	6	Comment: It is not indicated how risk categories should be assessed. Proposed change (if any): Please add a basic outline for criteria to assess whether	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		a Mab is a low, mid or high risk (or refer to an acceptable publication).	
277-283	7	Comment: It may be helpful here to define "Risk-based Approach". This term is sometimes misunderstood to simply mean the risk of developing an immune response. Proposed change (if any): We suggest adding: "The risk-based approach is an assessment of the potential for the patient to develop a drug-specific immune response combined with the potential for consequences of an induced immune response."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
277-283	8	**Comment:** It may be helpful here to define "Risk-based Approach." This term is sometimes misunderstood to simply mean the risk of developing an immune response. **Proposed change:** We suggest adding: **"<u>The risk-based approach is an assessment of the potential for the patient to develop a drug-specific immune response combined with the potential for consequences of an induced immune response</u>."**	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
280-293	7	Comment: The term "risk factors" is used throughout this section. We suggest removing the work "risk" as risk factors are patient and or product factors that are evaluated to identify risk.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): We suggest replacing "risk" factors with "patient" or "product" (as appropriate) factors that influence induction of immunogenicity. It may be helpful to add these as sub-headings within section 9.1.	
280-293	8	**Comment:** The term "risk factors" is used throughout this section. We suggest removing the work "risk" as risk factors are patient and or product factors that are evaluated to identify risk. **Proposed change:** We suggest replacing "risk" factors with "patient" or "product" (as appropriate) factors that influence induction of anti-drug antibodies. It may be helpful to add these as sub-headings within section 9.1.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
281	4	Comment: Risk factors may also include duration and frequency of treatment, concomitant medication and route of administration. Proposed change (if any): *"... final drug product and the treated patient population,* ***duration and frequency of treatment (acute / chronic), concomitant medication and route of administration.****"*	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
284	7	Comment: Please rephrase the term "immune response" as "the formation of anti-therapeutic antibodies" to make sure that only anti-drug antibody formation is discussed	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
284	11	Comment:	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Please rephrase the term "immune response" as "the formation of anti-therapeutic antibodies" to make sure that only anti-drug antibody formation is discussed	The clinical and risk sections have been redrafted taking these and other comments into account.
284	7	Comment: The term "Risk" may not be appropriate word here. Proposed change (if any): ~~Risk of~~ Potential for mounting an unwanted immune response.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
284	8	**Comment:** The term "Risk" may not be appropriate word here. **Proposed change:** ~~Risk of~~ **Potential for** mounting an unwanted immune response.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
284-327	9	Comment: These paragraphs contain information that appears to be pertinent to all protein biotherapeutics and is not unique to MAbs. The specific recommendations that are unique for mAbs are not clear. Proposed change: Delete sections that are redundant with general guidance and reference that guidance instead. Clarify what clinical consequences are unique to MAbs, what risks are/are not applicable to MAbs, and what specific actions should/need not be taken with regards to clinical evaluation of MAbs	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
284-327	10	Comment: These paragraphs contain information that appears to be pertinent to all protein biotherapeutics and is not unique to mAbs. The specific guidance with regard to mAbs is not clear Proposed change: See general comments In addition, please clarify what clinical consequences are unique to mAbs and what risks are/are not applicable to mAbs and what actions should/need not be taken with regards to clinical evaluation of mAbs.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
284-327	3	Comment: The whole of this section seems to have been added as an "after-thought" and appears to describe a number of general observations that are neither more nor less relevant to mAb products compared with other biopharmaceutical products. This information has already been provided in the general guideline. Proposed change (if any): Please delete this section, in favour of a cross-reference to the general guideline.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
284-327	5	Comment: It seems that all the way through section 9.1 the term "risk" is used, where actually "likelihood" would be more in compliance with the guidelines describing the risk based approach.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Please replace risk with likelihood or delete the whole section as it is similar to the general immunogenicity guidance (EMEA/CHMP/BMWP/14327/2006).	
284-237	7	Comment: It seems that all the way through section 9.1 the term "risk" is used, where actually "likelihood" would be more in compliance with the guidelines describing the risk based approach. In addition, the chapter discusses general considerations for protein drugs. Proposed change (if any): Please replace risk with likelihood or delete the whole section as it is similar to the general immunogenicity guidance.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
284-327	11	Comment: The chapter 9.2 discusses general considerations for protein drugs. Proposed change: Reduce to considerations for mAb therapeutics.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
285-286	4	Comment: A paragraph needs to be added to describe the approach for risk assessment for products undergoing clinical testing due to a process change and for biosimilars. Proposed change (if any):	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Adding the following paragraph: ***The assignment of the risk of unwanted immune responses for products after process changes or for biosimilars should be viewed in the context of the original molecule on a case by case basis. Because molecule specific immunogenicity risks are already known in these cases, a lower risk assignment than that for the first in human studies of the original mAb may be considered dependent upon the totality of the data.*** Comment: The general guide uses the following sorting for factors: patient, disease, and product-related risk factors. It would be helpful to use the same, uniform nomenclature since the guides are cross-referred. Proposed change (if any): *„This will depend on various factors that can be divided into ~~three~~ different subgroups, i.e.* ***patient-, disease- and product-related*** *risk factors (see general immunogenicity guideline).”*	
285-358	1	Comment: The risk discussion also suffers from an uncritical mixture of the obvious with highly speculative factors such as the role of impurities and host cell factors and the discussion of possible agonistic antibodies Proposed change (if any):	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Differentiate between facts and speculation	
285-358	5	Comment: In our opinion the risk discussion also suffers from an uncritical mixture of the obvious with highly speculative factors such as the role of impurities and host cell factors and the discussion of potential agonistic antibodies Proposed change (if any): We would propose to clearly indicate (e.g. by reference to literature) which immunogenicity risk factors are proven and to discriminate them from the ones that are rather based on speculation	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
291-293	7	Comment: Lines 291-293 make anintroductory statement for risk-based approach. Proposed change (if any): We suggest moving lines 291-293 up to section 9.0 as an introduction.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
291-293	8	**Comment:** Lines 291-293 make a good introductory statement for a risk-based approach. **Proposed change:** We suggest moving Lines 291-293 up to section 9.0 as an introduction.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
295	7	Comment:	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		"[...] to study immunogenic potential and measures implemented to potentially handle the clinical consequences [...]" Proposed change (if any): "[...] to study immunogenic potential and ~~measures~~ procedures implemented to potentially handle the clinical consequences [...]"	The clinical and risk sections have been redrafted taking these and other comments into account.
295	8	**Comment:** "[...] to study immunogenic potential and measures implemented to potentially handle the clinical consequences [...]" **Proposed change:** "[...] to study immunogenic potential and ~~measures~~ **procedures** implemented to potentially handle the clinical consequences [...]"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
299	7	Comment: Applies potentially also for products from mammalian cell lines. Proposed change (if any): Delete "non-mammalian products"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
299	11	Comment: Applies potentially also for products from mammalian cell lines. Proposed change: Delete "non-mammalian products"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
302	7	Comment: In this context, the word "possible" is too prescriptive.	Comment partly accepted. The clinical and risk sections have been redrafted taking these

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Levels of impurities can always be reduced, but at a cost, and often with little certainty of an incremental increase in safety. The words "reasonable and practical" may serve better here. Proposed change (if any): Replace "possible" to "reasonable and practical".	and other comments into account.
302	11	Comment: In this context, the word "possible" is too prescriptive. Levels of impurities can always be reduced, but at a cost, and often with little certainty of an incremental increase in safety. The words "reasonable and practical" may serve better here. Proposed change (if any): Replace "possible" to "reasonable and practical".	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
303	7	Comment: Examples for mAb product isoforms and degradation products could be provided and consequences for immunogenicity testing specified.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
303	11	Comment: Examples for Mab product isoforms and degradation products could be provided and consequences for immunogenicity testing specified.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
299-304	10	Comment: This section does not appear to be consistent with the risk based approach discussed earlier in the guidance. Acceptance criteria for impurities should be developed and justified, as	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		appropriate, recognizing that some impurities will have a limited immunogenic potential whereas others may be of greater concern. In addition, while some non-mammalian expression systems may produce known adjuvants, not all expression systems present the same potential for immunogenicity. Ultimately, this should all be considered in the risk assessment which would in turn establish appropriate targets for specific impurities.	
305-306	5	Comment: It should be mentioned that the immunogenicity risk associated with mAbs will often be low due to the lack of endogenous counterparts Proposed change (if any): Please revise accordingly	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
305-306	7	Comment: If section is retained, please explain how a mAb may have a high immunogenicity risk level although there is no endogenous counterpart. Proposed change (if any): The line 305 should be revised into: "At the beginning of clinical development applicants may have to apply a risk based approach ~~to assign a high risk level, although the mechanism of action may per se not necessarily suggest a higher risk~~	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
305-306	11	Comment: It is mentioned that "At the beginning of clinical development applicants may have to assign a high risk level, although the mechanism of action may per se not necessarily suggest a higher risk". A risk based assessment of immunogenicity should always be undertaken prior to entry into the clinic. Throughout the guideline, several considerations are mentioned as applicable to early clinical trials. But the regulatory position on early clinical development is not well defined. Will EMA issue a separate guideline on immunogenicity assessment in early clinical development? Proposed change (if any): Please clarify how this guideline does (or does not) relate to early clinical development.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
305-308	7	Comment: It is unclear whether the risk assessments performed early in clinical development should be communicated to the regulatory agencies. Proposed change (if any): Add more description of how the agency would like the sponsors to document and communicate risk assessments during a clinical development program. Also suggest adding more clarity around what is considered low versus high risk, perhaps with examples.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
305-308	7	Comment: lines 305-308 are introductory topics for risk-based approach. Proposed change (if any):	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		We suggest moving lines 305-308 up to section 9.0 as an introduction and then using "product factors" and "patient factors" as sub-headings within the text of section 9.1 to organize the topics to the reader.	
305-308	8	**Comment:** Lines 305-308 are good introductory topics for a risk-based approach. **Proposed change:** We suggest moving Lines 305-308 up to section 9.0 as an introduction and then using "product factors" and "patient factors" as sub-headings within the text of section 9.1 to organize the topics to the reader.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
309-317	10	Comment: Whilst the route of administration may be associated with different immunogenicity profiles, other drug, formulation and patient factors may be more significant. Therefore, instead of classifying differing routes of administration as 'lower, medium, and higher', it may be more appropriate to simply refer to the language in the existing guideline – "Products given intravenously may be less immunogenic than those given subcutaneously or intramuscularly." Please provide clarification with regards to 'long-term treatment' and 'the optimal time period between repeated administrations'. This is determined on the basis of the PK/PD relationship for each mAb.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
311	7	Comment: There are publications indicating that e.g. the i.m. route	Comment partly accepted. The clinical and risk sections have been redrafted taking these

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		of application is more immunogenic than the s.c. route of application. Proposed change (if any): Please delete the sentence.	and other comments into account.
315-316	7	Comment: There are publications which show the opposite. Proposed change (if any): Please delete	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
315-316	11	Comment: There are publications which show the opposite. Proposed change (if any): Please delete the sentence "In general, short-term treatment is usually associated… than long-term treatment"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
315-317	8	**Comment:** The recommendation in these sentences could be interpreted to mean that an applicant should determine the effect of dosing interval on unwanted immune response. Given the rare nature of many immune responses and the dependence of the immune response on various patient and disease state related factors, this interval would be impossible to predetermine. **Proposed change:** In general, short-term treatment is usually associated with a lower risk of inducing an unwanted immune response than long-term treatment.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		~~For the latter, the optimal time period between repeated administrations should be determined.~~	
316-317	9	Comment: The guideline states that short-term treatment is usually associated with a lower risk of inducing an immune response than long-term treatment and that the optimal time period between repeated administrations in long-term treatment should be determined. This could be interpreted to mean that the effect of the dosing interval on the immune response should be determined. This recommendation is not practical. Calculating the dose and frequency of administration that results in the lowest level of immunogenicity can require large clinical trials over a lengthy period of time. Even then, they would be nearly impossible to calculate given the rare nature of many immune responses and the dependence of immune response on various patient and disease state related factors. Proposed change: Delete sentence on lines 316-317.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
316-317	2	Comment: The draft guideline recommends that for mAbs intended for long-term treatment, applicants should determine the optimal time period between repeat administrations. In this context, such a recommendation could be interpreted to mean that an	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		applicant should determine the effect of dosing interval on unwanted immune response. This suggestion may be impractical. Calculating the dose and frequency of administration that results in the lowest level of immunogenicity can require large clinical trials over a lengthy period of time. Even then, they would be nearly impossible to calculate given the rare nature of many immune responses and the dependence of immune response on various patient and disease state related factors. In any case, PK, efficacy, and safety are often more important drivers of dosing intervals. We recommend that the sentence on lines 316-317 be deleted. Proposed change (if any): "~~For the latter, the optimal time period between repeated administrations should be determined.~~"	
316-317	7	Comment: Lines 316-317 currently recommend that applicants determine the "optimal time" between doses for mAbs intended for long-term treatment. The exact meaning of this statement is unclear, but it could refer to determining the effect of dosing schedules on unwanted immunogenicity. It will generally be very difficult for an applicant to determine what administration schedule will result in the lowest level of immunogenicity. This type of determination would require massive long-term clinical trials. Any data gathered from these trials could be difficult to interpret (i.e., to use in calculating the	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		optimal administration schedule) because of the many different patient-related and product-related factors that affect immunogenicity. Treatment durations in clinical trials are selected based upon many factors. Immunogenicity is one of these factors, but it is typically not the primary consideration. This sentence implies that the optimal time period between repeated administrations is largely driven by immunogenicity data. In reality, few trials are designed where the dosing regimen has been optimized to minimize ATA incidence. The sentence should be deleted. Additionally, consider specifying how the optimal time between administrations could be determined. Proposed change (if any): Please clarify, or delete the following sentence: ~~For the latter, the optimal time period between repeated administrations should be determined.~~	
316-317	11	Comment: "In general, short-term treatment is usually associated with a lower risk of inducing an unwanted immune response than long-term treatment. For the latter, the optimal time period between repeated administrations should be determined." Treatment durations in clinical trials are selected based upon many factors. Immunogenicity is one of these factors, but it is typically not the primary consideration. This sentence implies that the optimal time period between repeated administrations is largely driven by immunogenicity data. In reality, few trials are designed	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		where the dosing regimen has been optimized to minimize ATA incidence. Additionally, consider specifying how the optimal time between administrations could be determined. Proposed change (if any): Please clarify	
316, 328, 330	7	Please rephrase the term "immune response" to "the formation of anti-therapeutic antibodies" to clarify that formation of anti-drug antibodies is discussed	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.*)*
316, 328, 330	11	Please rephrase the term "immune response" to "the formation of anti-therapeutic antibodies" to clarify that formation of anti-drug antibodies is discussed	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
318-322	7	Comment: The potential difference in immunogenicity of mAbs between adults and children needs to be evaluated in the context of clinical relevance and scientific appropriateness. Proposed change (if any): After "Extrapolation of immunogenicity data from a previously conducted clinical study in adults is not sufficient", we suggest adding: "However, if the potential for differences between adults and children is not scientifically reasonable or clinically relevant, it would be considered appropriate to use adult	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		immunogenicity data as supportive with limited evaluation if required in children and this should be discussed with the Agency on a case by case basis." Comment: "Children may have higher protein metabolism and a different immune status than adults, and cases are known where data suggest a considerably higher immunogenicity of mAbs. In this patient group immunogenicity should be evaluated separately as for adults. Extrapolation of immunogenicity data from a previously conducted clinical study in adults is not sufficient." We agree that immunogenicity data from adults should not be extrapolated to children, and that, for some mAbs, the ATA incidence may be higher in children than in adults. Consider addition that due to the group size it may not be possible to carry out statistically relevant evaluation on the children group Proposed change (if any): Please more explicitly state that immunogenicity data from adults should usually not be extrapolated to children.	
318-322	11	Comment: "Children may have higher protein metabolism and a different immune status than adults, and cases are	Comment partly accepted. The clinical and risk sections have been redrafted taking these

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		known where data suggest a considerably higher immunogenicity of mAbs. In this patient group immunogenicity should be evaluated separately as for adults. Extrapolation of immunogenicity data from a previously conducted clinical study in adults is not sufficient." We agree that immunogenicity data from adults should not be extrapolated to children, and that, for some mAbs, the ATA incidence may be higher in children than in adults. Consider addition that due to the group size it may not be possible to carry out statistically relevant evaluation on the children group Proposed change (if any): Please more explicitly state that immunogenicity data from adults should usually not be extrapolated to children.	and other comments into account.
324	7	Comment: It is proposed to include the immune status of the patients into risk assessment "Please specify which aspects of patient immune status should be taken into account".	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
324	11	Comment: It is proposed to include the immune status of the patients into risk assessment "Please specify which aspects of patient immune status should be taken into	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		account".	
326-327	2	Comment: Section 9.1 of the guideline discusses the risk of mounting an unwanted immune response. The final sentence ("The risk perception may be higher if the methodology to either detect anti-drug antibodies or to detect clinical consequences is not sensitive.") seems out of place. The use of insensitive methodology does not affect the risk of mounting an unwanted immune response as this sentence appears to imply. Such methodology may, however, affect patient safety risks more broadly given its limited ability to anticipate and/or detect an unwanted immune response. Proposed change (if any): The CHMP should delete the sentence on lines 326-327 or modify it for clarity.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
326-327	6	Comment: Please explain the statement "The risk perception may be higher..": The perception is higher than what?	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
326-327	7	Comment: The sentence on lines 326-327 is in a section entitled risk of mounting an unwanted immune response," but is more about the ability to detect a response than about the risk of mounting one. The draft guideline elsewhere adequately addresses the need to use sensitive testing methodologies, so this statement should be removed.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Alternatively, the CHMP could clarify that suboptimal methodologies do not affect the risk of unwanted immunogenicity, but they do affect an applicant's ability to anticipate or detect such a response. Proposed change (if any): Lines 236-327 should be deleted	
326-327	7	Comment: This statement appears out of place here and is not a clear summary statement. Proposed change (if any): We suggest moving lines **326**-327 into introduction section 9.0.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
326-327	8	**Comment:** This statement appears out of place here and is not a clear summary statement. **Proposed change:** We suggest moving Lines 326-327 into introduction section 9.0.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
326-327	9	Comment: In the discussion of the risk of mounting an unwanted immune response (Section 9.1), the final sentence ("The risk perception may be higher if the methodology to either detect anti-drug antibodies or to detect clinical consequences is not sensitive.") seems out of place. The use of insensitive methodology per se does not affect the risk of mounting an unwanted immune	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		response as this sentence appears to imply. Such methodology may, however, affect patient safety risks more broadly given its limited ability to detect an unwanted immune response. Proposed change : Delete lines 326-327 or modify for clarity	
328	7	Comment: We suggest rewording the title to provide a range of impact from "not severe" to "severe". Proposed change (if any): "The ~~severity~~ impact of clinical consequences of an immune response"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
328	8	**Comment:** We suggest rewording the title to provide a range of impact from "not severe" to "severe." **Proposed change:** "The ~~severity~~ **impact** of clinical consequences of an immune response"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
328-358	3	Comment: Again, this information belongs at the front of the guideline, to help guide Applicants in developing a product-specific strategy to identify (and address) the pertinent risk factors. This is a somewhat long-winded text that obscures the critical messages.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change (if any): Please see recommendations above for lines 42-58. Effectively, these cover the points made in the draft guideline text lines 328-358, but rather more succinctly.	
329-332	6	Comment: Does EMA expect regular communication on a scientific base with the sponsor?	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
333-336	7	Comment: Please add the scientific citation	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
333-336	11	Comment: Please add the scientific citation	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
336	7	Comment: "Non-idiotypic antibodies to mAbs can be clinically important by positively or negatively affecting the bioavailability of the product." The word "bioavailability" can be replaced by "systemic exposures". What is changed by antibodies is more likely AUC, exposure, or disposition rather than "bioavailability". Disposition refers to distribution and elimination while bioavailability refers to extent of mAbs	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		reaching circulation from SC or IM depot. A wide range of SC bioavailability values has been observed for mAbs, while a good correlation between bioavailability and (non-idiotypic) immunogenicity has not been established. Proposed change (if any): Please rewrite, e.g. to: "Non-idiotypic antibodies to mAbs can be clinically important by positively or negatively affecting the systemic exposures of the product."	
336	11	Comment: "Non-idiotypic antibodies to mAbs can be clinically important by positively or negatively affecting the bioavailability of the product." The word "bioavailability" can be replaced by "systemic exposures". What is changed by antibodies is more likely AUC, exposure, or disposition rather than "bioavailability". Disposition refers to distribution and elimination while bioavailability refers to extent of mAbs reaching circulation from SC or IM depot. A wide range of SC bioavailability values has been observed for mAbs, while a good correlation between bioavailability and (non-idiotypic) immunogenicity has not been established. Proposed change (if any): Please rewrite, e.g. to: "Non-idiotypic antibodies to mAbs can be clinically important by positively or	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		negatively affecting the systemic exposures of the product.”	
337-338	10	Comment: What clinical effects similar to those mediated by rheumatoid factors have actually been observed by non-idiotypic antibodies to mAbs, regarding actual clinical events or laboratory tests, e.g., interference in PK or ADA assays?	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
337-339	7	Comment: Please add references or describe the clinical effect mediated by rheumatoid factors Proposed change (if any): Please see comment	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
337-340	7	Comment: Please specify the meaning of the statement, currently not clear. Please explain the effects mediated by rheumatoid factors. Please provide relevant citations for the phenomenon of epitope spreading and its relevance for mAb therapeutics.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
337-340	11	Comment: Please specify the meaning of the statement, currently not clear. Please explain the effects mediated by rheumatoid	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		factors. Please provide relevant citations for the phenomenon of epitope spreading and its relevance for mAb therapeutics.	
340	7	Comment: Suggest to use affinity maturation instead of epitope spreading Proposed change (if any) see comment	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
342	7	Comment: Immunological and pharmacological consequences of the presence of ATAs may differ between individual mAb therapeutics. Proposed change (if any): Add this statement	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
342	11	Comment: Immunological and pharmacological consequences of the presence of ATAs may differ between individual mAb therapeutics. Proposed change (if any): Add this statement	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
346	7	Comment: Notably, first-infusion reactions are often not triggered by ADA but rather depend on the pharmacological function of the mAb product and involve other immune parameter, such as cytokines release and complement activation.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
346	11	Comment: Notably, first-infusion reactions are often not triggered by ADA but rather depend on the pharmacological function of the mAb product and involve other immune parameter, such as cytokines release and complement activation.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
347-348	7	Please rephrase "Potentially threatening cytokine release syndromes" to "potentially resulting in cytokine storm"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
347-348	7	Please rephrase "Potentially threatening cytokine release syndromes" to "potentially resulting in cytokine storm"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
352-358	8	**Comment:** Although important, the examples provided in this paragraph cannot be predicted and cannot be taken into consideration during risk-based approach. This statement is more appropriate in the "Clinical Consequences" section. **Proposed change:** We suggest moving this paragraph to the "Clinical Consequences" section 6, Lines 158-162.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
352-358	7	Comment: Although important, the examples provided in this paragraph cannot be predicted and cannot be taken into consideration during risk-based approach. This statement is more appropriate in the "Clinical	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Consequences" section. Proposed change (if any): We suggest moving this paragraph to the "Clinical Consequences" section 6, lines 158-162. Comment: The clinical decision following a hypersensitivity reaction is not dependent on the technical ATA assay result. In addition, the section is not specific for mAb therapeutics. Proposed change (if any): Delete paragraph	
354-355	7	Comment: In our experience the immunogenicity is increased when the time interval between doses is increased. This is in agreement with common knowledge, when attempting to raise antibodies in animals. Proposed change (if any): "The incidence of such unwanted immune responses is also dependent on the time interval between doses and usually ~~reduces~~ increases with longer time periods."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
352-358	11	Comment: The clinical decision following a hypersensitivity reaction is not dependent on the technical ATA assay result. In	Comment partly accepted. The clinical and risk sections have been redrafted taking these and

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		addition, the section is not specific for mAb therapeutics. Proposed change: Delete paragraph.	other comments into account.
359	7	Comment: The content within section 9.3 is not necessarily the "consequences with regard to risk classes" as much as it is the considerations for characterizing an induced immune response based upon the risk level determined in the risk assessment. Proposed change (if any): "~~Consequences with regard to different risk classes~~" "Risk level-dependent characterization of immune response"	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
359	8	**Comment:** The content within section 9.3 is not necessarily the "consequences with regard to risk classes" as much as it is the considerations for characterizing an induced immune response based upon the risk level determined in the risk assessment. **Proposed change:** "~~Consequences with regard to different risk classes~~" "**Risk level-dependent characterization of immune response**."	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
359-375	3	Comment: This section is very difficult to understand and should be deleted.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		The most important criteria for defining consequence are the clinical indices, not the results of bioanalytical assays. Thus, the logic of the first sentence is difficult to understand. The second paragraph compounds a number of qualifying statements, introduced by the words "however", "depending" and "nevertheless", which confuse rather than clarify. The final paragraph does not provide any guidance on what should be included in the Risk Management Plan. Proposed change (if any): The consequences of the undesirable immunogenicity of mAb products should be determined by reference to relevant clinical indices, including PK, PD, efficacy and safety parameters. The Applicant should correlate the results of bioanalytical assays with these clinical parameters in order to justify the suitability of the methods used. As with other biopharmaceutical products, the consequences of the immunogenicity of mAb products should be evaluated in the wider population. If clinically-significant immunogenicity is suspected as a causal factor for loss of therapeutic response of for the appearance of adverse events, it is important to investigate the nature of the immune response in affected subjects to confirm or eliminate the role of a host immune response. For example, it may be	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		necessary for a Marketing Authorisation Holder to provide a suitable assay to enable clinicians to measure ADA levels in treated subjects. In the case of suspected Type 1 hypersensitivity reactions, *ex-vivo* challenge of basophils with the mAb product might be considered as the most relevant diagnostic method.	
359-375	7	Comment: This important chapter should guide applicants for mAb therapeutics - especially in its differences to other protein therapeutics. Proposed change (if any): Add specific consequences for mAb development	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
359-375	11	Comment: This important chapter should guide applicants for mAb therapeutics - especially in its differences to other protein therapeutics. Proposed change (if any): Add specific consequences for mAb development	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
360-361	8	**Comment:** This sentence suggests that a neutralizing antibody assay is required for all mAbs that are confirmed positive, without exceptions or alternative approaches for assessing neutralizing antibodies. **Proposed change: "**For all mAbs a validated screening and confirmatory assay should be performed, ~~followed by a validated neutralizing assay in case of positive~~	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		~~results in the confirmatory assay~~ **and the neutralizing potential of confirmed drug-specific antibodies should also be evaluated with a neutralizing antibody assay or acceptable alternative**."	
360-361	4	Comment: It is questionable whether there is value in testing low titre antibodies Proposed change (if any): *"For all mAbs a validated screening and confirmatory assay should be performed followed by a validated neutralizing assay in case of **high titre** positive results in the confirmatory assay."*	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
360-361	7	Comment: This sentence suggests that a neutralizing antibody assay is required for all mAbs that are confirmed positive. Evaluation of a relevant and sensitive PD marker can sometimes effectively determine neutralisation of drug. Proposed change (if any): "For all mAbs a validated screening and confirmatory assay should be performed and the neutralizing potential of confirmed drug-specific antibodies should also be evaluated with a neutralizing antibody assay or acceptable alternative	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
360-361	11	Comment: The need for a NAb assay may not be warranted in early clinical development, and based upon the risk	Comment partly accepted. The clinical and risk sections have been redrafted taking these and

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		assessment (sections 9.1, 9.2) could reasonably be delayed until pivotal trials. Proposed change (if any): Please see also other comments on Nab assay implementation.	other comments into account.
360-364	6	Comment: • Please explain why it is essential to distinguish between neutralising and non-neutralising regardless of their risk level? Be more specific. • For low risk mAb's a screening assay with a cutpoint of 99 or 99.9% false positive in Phase I & II may also suffice. • Earlier in the document (see lines 252 to 260), the use of "competitive ligand binding assays" to determine drug specific neutralizing antibodies is proposed as adequate assays to assess "neutralizing antibodies". Here it is stressed that the use of an "neutralizing assay" is necessary. This seems in contradiction with the earlier statement. Proposed change (if any): Please consider the last suggestion on cutpoint % levels.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
361-	8	**Comment:** Non-neutralizing antibodies can also affect efficacy and safety. An increase in clearance (caused by non-neutralizing antibodies) can have as profound	Comment partly accepted. The clinical and risk sections have been redrafted taking these

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		an impact on efficacy as neutralization. In addition, it is currently unclear whether neutralizing and non-neutralizing antibodies pose different risks relating to infusion or injection site reactions, a common adverse event associated with mAb therapies. **Proposed change: "**Distinguishing between neutralizing and non-neutralizing antibodies is essential for all mAbs, regardless of their risk level, ~~as lack of, or even reduced efficacy due to the neutralizing activity of the antibodies may result in a discontinuation of treatment with the mAb.~~ **to identify potential mechanisms of impact on safety and efficacy."**	and other comments into account.
361-364	2	Comment: The draft guideline reasons that distinguishing between non-neutralizing and neutralizing antibodies is critical because of the safety risks or reduced efficacy that can result from neutralizing antibodies. Such a statement minimizes the impact of non-neutralizing antibodies, which can also affect safety and efficacy. Indeed, an increase in clearance (caused by non-neutralizing antibodies) can have as profound an impact on efficacy as neutralization. In addition, regardless of neutralizing status, antibodies may pose risks related to infusion or injection site reactions. Proposed change (if any): "Distinguishing between neutralizing and non-neutralizing antibodies is essential for all mAbs	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		regardless of their risk level **to help identify potential mechanisms of impact on safety and efficacy.**~~as lack of, or even reduced efficacy due to the neutralizing activity of the antibodies may result in a discontinuation of treatment with the mAb~~.”	
361-364	7	Comment: In the case of an antibody response to a human or humanized therapeutic mAb which is usually anti-idiotypic, assessment of neutralizing activity could be characterized and verified in initial studies if needed. The most relevant assessment of neutralizing activity is the correlation of ATA development with loss of efficacy in a clinical trial. The draft guideline currently states that distinguishing between non-neutralizing and neutralizing antibodies is critical given the serious consequences that neutralizing antibodies can cause in terms of reduced efficacy. This concern, however, is not unique to neutralizing antibodies. Non-neutralizing antibodies can similarly result in changes in safety and efficacy, for example, by altering clearance of the mAb. Proposed change (if any): Distinguishing between neutralizing and non-neutralizing antibodies is essential for all mAbs regardless of their risk level in order to help categorize all potential immunogenic reactions that can affect safety and efficacy	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Comment: The presence of Nabs coincident with loss of efficacy is not always correlated with cause/effect--a large dataset is needed to derive that relationship. Further, as pointed out earlier in this guidance, ATAs can be transient so that depending on the risk-assessment, which includes disease severity as well as therapeutic alternatives, discontinuation based upon the presence of Nabs may be unwarranted and potentially remove beneficial treatment from patients prematurely. Subjects with Nabs should be monitored so appropriate intervention including cessation of therapy can be performed if necessary. Proposed change (if any): The guidance should acknowledge that the presence of Nabs and loss of efficacy are not always causally related.	
361-364	11	Comments: Development of NAb assays should be risk based, and done at an appropriate phase during a mAb development program rather than a default procedure for all mAbs. A distinction between Nab and binding Abs may be of no or only limited clinical relevance depending on risk defined by the actual mAb product. In addition, assay technologies used for binding and neutralizing	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		antibodies usually have different performance characteristics, and, therefore the mere absence of neutralizing activity does not mean none exists (especially if the sensitivity of the Nab assay does not match the sensitivity of the screening assay). Nab assay results may therefore have limited relevance and would be dispensable in certain cases. Please acknowledge this consideration and provide guidance when a Nab assay may be dispensable. Proposed change: In the absence of clinical events that are associated with antibody positive samples, distinguishing between Nab and non-Nab may not be relevant. Please clarify.	
361-364	9	Comment: The draft guideline reasons that distinguishing between non-neutralizing and neutralizing antibodies is critical because of the safety risks or reduced efficacy that can result from neutralizing antibodies. Non-neutralizing antibodies, however, can also affect efficacy and safety. Indeed, an increase in clearance (caused by non-neutralizing antibodies) can have as profound an impact on efficacy as neutralization. In addition, it is currently unclear whether neutralizing and non-neutralizing antibodies pose different risks relating to infusion or injection site reactions, a common adverse event associated with mAb therapies.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Proposed change: Amend lines 361-364 as follows: "Distinguishing between neutralizing and non-neutralizing antibodies is essential for all mAbs regardless of their risk level to help identify potential mechanisms of impact on safety and efficacy."	
362-364	4	Comment: As mentioned also in this guideline, reduced efficacy or lack of response may result also from non-neutralising antibodies by faster clearance/metabolisation of the mAb, dependent on the antibody titer and affinity to the (non-neutralising) epitope, which leads to high complex building. Proposed change (if any): Add the following sentence at the end of Line 364: ***"However, reduced efficacy or lack of response may result also from non-neutralising antibodies by faster clearance/metabolisation of the mAb, dependent on the antibody titer and affinity to the (non-neutralising) epitope ".***	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
362-364	7	Comment: It is not clear why it is essential to distinguish between neutralising and non-neutralising regardless of their risk level? Further explanation would be useful.	Comment partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
365-371	6	Comment: "Banking of samples" may not be in Compliance with GCP regulations. Please consult your draft GCP reflection paper (EMA/INS/GCP/532137/2010).	The clinical and risk sections have been redrafted taking these and other comments into account.
369-370	2	Comment: There is a reference to routine banking of samples during a mAb's development program, but the guideline does not provide any additional details on how this should be accomplished. Proposed change (if any): We request that the CHMP clarify its expectations regarding how the banking and testing of samples should be handled.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
369-370	7	Comment: The draft guideline recommends that applicants routinely bank samples taken during a mAb's development program. The CHMP should include additional detail in the guideline regarding its expectations on how the banking of samples should be accomplished.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
369-370	9	Comment: There is a reference to routine banking of samplesduring a mAb's development program. We request that the CHMP clarify its expectations regarding how the banking and testing of samples should be	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		handled. Proposed change: Clarify expectations for banking and testing of samples.	
371	4	Comment: Real time may not be the best word. For high risk mAbs, it is proposed to analyse blood samples more frequently in order to identify any risks as early as possible. However, from a practical point of view, this should be performed with caution to avoid introduction of higher inter-assay variability. Therefore, we recommend rephrasing this paragraph to highlight the problems associated with frequent testing. Proposed change (if any): *"In this situation it is advisable to ~~analyze samples in real time~~* ***discuss with the Regulatory Agency the frequency of batch testing of samples on a case-by-case basis to identify the most optimal frequency for generating reliable results while ensuring adequate patient safety."***	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
371	7	Comment: Please define "frequent" Proposed change (if any): Insert explanation	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Comment: Definition of "real time" is unclear. Proposed change (if any): Clarifying the meaning of "real time"	
372	7	Comment: " The approach outlined above [...]". It is not clear what approach this statement refers to. Proposed change (if any): We suggest clarifying the above statement	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.
372	8	**Comment:** "The approach outlined above [...]". It is not clear what approach this statement refers to. **Proposed change:** We suggest clarifying the statement above.	Comment partly accepted. The clinical and risk sections have been redrafted taking these and other comments into account.