European Medicines Agency
Pre-Authorisation Evaluation of Medicines for Human Use

London, 18 December 2008
Doc. Ref.: EMEA/CHMP/BWP/651330/2008

OVERVIEW OF COMMENTS RECEIVED ON THE GUIDELINE "DEVELOPMENT, PRODUCTION, CHARACTERISATION AND SPECIFICATIONS FOR MONOCLONAL ANTIBODIES AND RELATED PRODUCTS"

Table 1: Organisations that commented on the draft Guideline as released for consultation

	Name of Organisation or individual	Country
1	European Federation of Pharmaceutical Industries and Associations (EFPIA) / European Biopharmaceutical Enterprises (EBE)	Belgium
3	Hungarian Academy of Sciences (HAS) – Immunological Committee	Hungary
4	Laboratoire du Fractionnement et des Biotechnologies (LFB)	France
5	Lonza Biologics plc (LONZA)	The United Kingdom
6	Ludger Ltd (LUDGER)	The United Kingdom
7	MedImmune Ltd (MEDIMMUNE)	The United Kingdom
8	Merck Sharp & Dohme (Europe) Inc. (MSD)	Belgium
9	Parenteral Drug Association (PDA)	Germany
10	Roche Group of Companies (ROCHE)	Switzerland
11	Wyeth Research (WYETH)	France

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 16
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

Table 2: Discussion of comments

GENERAL COMMENTS - OVERVIEW
EFPIA/EBE: EFPIA/EBE members welcome the proposal to revise the guideline in line with industry and agency experience gained since the issue of the existing guidance document, Production and Quality Control of Monoclonal Antibodies (3AB4a), July 1995. In general, the proposed revisions are welcomed. However, EBE members have significant concerns related to the alignment of this proposed guidance with existing ICH and regional guidelines, and the nature of specific requirements. Alignment to Existing Guidance In consideration of the number of monoclonal antibody products currently in development and the specific aspects of the production and quality control of products in this class, it is felt that the guidance would benefit from discussion within a global setting. EBE members would encourage the BWP to bring this topic forward into the ICH arena so that agreement can be reached on requirements across the 3 major regions, and inconsistencies with existing ICH and regional guidelines can be resolved. Flexibility of Requirements It is felt that certain aspects of the guidance are unduly prescriptive and would limit the application of a risk based approach to process control and product quality. This is contrary to the principles outlined in ICH Q8, Q9 and Q10, and is therefore of significant concern. In particular, it is felt that the focus of specification setting should be on the testing of critical quality attributes of the antibody rather than on process consistency, which should be assured by means other than end product testing. Monoclonal antibodies cannot be considered a single class of molecule as regards the relationship between specific quality attributes, and it is therefore inappropriate to set blanket specifications. For instance, it is considered that the requirement to establish specifications for monitoring glycosylation on a routine basis may not be scientifically justified in all cases. Throughout the document there is no distinction between neutralizing antibodies and those with effector function with respect to the importance of glycosylation and characterization of the glycans (for example, see page 9, lines 12-13. "All glycan structures present should be fully characterized, paying attention to the degree of sialylation" and page 10 lines 45-47 "a specification for glycosylation should *always* be set"). If the antibody's mode of action is not dependent upon effector function and consistency of glycosylation can be demonstrated, a specification should not be necessary. We request this option is included in the guidance for neutralizing antibodies. See specific comments for Section 4.5.3. A further example of this relates to the requirements for formulation development and specifications for particulate matter. As drafted, the guidance does not make a distinction between extrinsic particulate matter and intrinsic proteinaceous particles, stating that particles are unwanted, their formation should be avoided through appropriate formulation development, and lots should be tested according to the Ph Eur requirements for visible and sub-visible particles. Whilst it is clearly the aim of formulation development to deliver formulations that are not subject to aggregation and/or particle formation, the factors leading to particle formation are not always understood, and low levels of particles may be present in formulations, despite significant efforts on the part of the manufacturer. It is important that particulate matter is identified, characterised by state of the art methods, and that the kinetics of formation are understood. Where this assessment indicates that the presence of particles does not pose risks to product safety and efficacy, then this information should be sufficient to support approval. As stated, the guidance implies an absolute prohibition for the presence of particles, which may have the effect of delaying patient access to important new therapies. This is not considered to be an overstatement of the potential impact of the proposed requirement given that other approved products are known to contain intrinsic proteinaceous particles.

HAS:
The draft guideline may fulfil the aim to provide guidance on the specific quality issues concerning monoclonal antibodies beyond the requirements, which apply to any therapeutic protein. The guideline should focus on the most frequently used current technologies and product variants leaving space to the upcoming developments (phage display, transgenics) as well.

LUDGER:
Overall, this is a good document. There are important things missing from the sections on glycosylation of antibodies.

MEDIMUNE:
The Guidance makes insufficiently clear distinction between products at an early stage of development and those approaching licensure. Our general comment would be that information and knowledge about the production and quality control systems develops with the development of the product, and that information should be provided that is appropriate to the phase of development and knowledge of the manufacturing system, with due regard for patient safety. Therefore, there is a need for more specific guidance related to phase of development, being mindful that excessive early information burden will slow down innovative product development and may not be appropriate.
There is inconsistency in the scope of information required. It is clear that this guideline is intended to describe requirements specific for monoclonal antibodies, but much of the information is also valid for all recombinant products. It should be made clearer where the guidance is specific to monoclonal antibodies. In other cases, a method is proposed but the data specifically asked for is not defined.
Heterogeneity will be characterized during development of the product and process, as described in ICH Q6B. The risk to safety and efficacy from heterogeneity may be evaluated using a risk assessment process (ICH Q9). The risk can be reduced by establishing a design space, as described in ICH Q8.

MSD:
The guidance is generally acceptable. The majority of the comments made are intended to eliminate potential ambiguity that may lead to misinterpretation of the guidance.

PDA:
The PDA expert committee used the following criteria for preparing our comments:
• The guidance is generally applicable for all monoclonal antibodies (Mabs) and related substances,
• The guidance should include advice to facilitate new technologies and innovative products – both current and future focus,
• The scope of the guidance is strictly for products at the marketing stage in order to facilitate the information in a Marketing Authorisation Application. (The scope does not include IMPs/clinical trial materials).
• The scope of the guidance is for manufacturing and QC aspects only. (The scope does not include aspects unrelated to manufacturing, e.g. epitope determination and cross-reactivity.)
The document covers the requirements for the contents and approach for a Marketing Authorisation application. Care should be taken to keep specific approaches to a minimum in order to facilitate development of new and innovative products and processes. We appreciate the incorporation of provisions for platform manufacturing in this draft guidance and see this as a first step in beginning to incorporate some of the principles of Quality by Design (QBD). However, we would like to see this guidance take the next step and incorporate further QBD principles from ICH Q8, Q9, and Q10. For instance, specifications should be driven by critical quality attributes (parameters critical to the safety and or efficacy of the molecule). Also, this guidance should address potential for regulatory flexibility for sponsors who provide detailed knowledge supporting design space in the application.

To facilitate innovative technologies and products, and to avoid confusion, several references and terminologies should be modified and, references to other specific and relevant Directives, Guidances and GMPs should be made.

For terminology and definitions the following approaches are recommended:

(1) The term "related substances" is used too broadly throughout the document in relation to an antibody component in the constant or variable region of the molecule. This term should be reserved solely for the definition in ICH Q6B. In this guideline, "antibody derived products" or "antibody- related protein" may be more appropriate terminology. Under the ICH Q6B definition, related substances are "variants of a desired product that are formed during the manufacturing process that have properties comparable to the desired product". We suggest the term "Related Products" may be more appropriate for use in the title. (Changes need to be made in numerous places in the document including: in the title, Intro line 18, 26 and 4.1 line 22 and section 4.4 line 18, etc.).

(2) Throughout the document there is no distinction between neutralizing antibodies and those with effector function with respect to the importance of glycosylation and characterization of the glycans (for example, see page 9, lines 12-13, *"All glycan structures present should be fully characterised, and although most antibodies are not sialylated when they are, by paying attention to the degree of sialylation*" and page 10 lines 45-47 *"a specification for glycosylation should always be set"*). If the antibody's mode of action is not dependent upon effector function and consistency of glycosylation can be demonstrated, a specification should not be necessary. We request this option is included in the guidance for neutralising antibodies. See specific comments for Section 4.5.3.

PDA/ROCHE:

(3) In the introduction, a description is given differentiating between murine, chimeric, humanised and fully human monoclonal antibodies. These terms relate to the protein component of the antibodies only. It is worth noting that the glycosylation of Mabs is determined by the host cells used for expression in cell culture. The expressed Mabs are not necessarily human-like, which are always fucosylated. Nevertheless, technologies are being developed which provide glycosylated Mabs, possessing non-fucosylated oligosaccharides. These modified Mabs have a higher affinity for the human FcgRIIIa receptor on immune effector cells, which can potentially lead to more efficient antibody-dependent cellular cytotoxicity (ADCC).

(4) In general, the document should move away from the non-human, chimeric and human descriptions as shorthand regarding the immunogenicity of a monoclonal antibody. The understanding of the role of T cell epitopes and the ability to engineer those epitopes should be stressed.

(5) Differences between this Monoclonal Antibody draft guideline and the guidelines on the production and control of rDNA products should be specifically mentioned or discussed.

WYETH:

In general, Wyeth believes the draft guideline is a well-written document. However, the regulatory concerns behind particular requirements/recommendations have been specified in many but not all cases. It would be beneficial for the manufacturer to know (to the extent possible) if there are any additional concerns/considerations that could be addressed by process and product development and characterisation or by quality control. These concerns may be applicable to all molecules covered in the guideline or to a specific class only.

Comments:

Following the public consultation for the guideline on monoclonal antibodies (Mab) (EMEA/CHMP/BWP/157653/2007), the Biologics Working Party Drafting Group reviewed the comments received and significantly revised the document. Several points required further discussion with interested parties, and thus, scientific experts from European trade associations (EBE/EFPIA, EuropaBio and PDA) participated to a meeting at EMEA on 18 June 2008 to discuss:

- ***Terminology to be used in the guideline to refer to products related to monoclonal antibodies.***
- ***Need to describe in the guideline the differences for IgG, IgM, IgE, fragments and fusion proteins,***
- ***Flexibility of requirements and aspects relating to the "platform manufacturing" approach, including viral safety data.***
- ***Need to describe specific analytical methods in the guideline.***
- ***Setting of specifications including glycosylation, C-terminal heterogeneity and process-related impurities.***
- ***Requirements regarding the presence of visible and sub-visible particulates in the drug product.***

The outcome of the discussions on these different points are reflected in following sections of this document..

The need for an ICH harmonisation of this topic has been acknowledged, however, this should be further discussed at ICH level, and for the time being, the current EU document is outdated, and required a revision.

To avoid confusion with the definition of "product related substance", as described in ICHQ6B, it was agreed during the above-mentioned meeting Industry/BWP that the terms "Mab-related substance" should be replaced by "Mab-related product".

SPECIFIC COMMENTS ON TEXT

GUIDELINE SECTION TITLE

Page no. + Line no.	Comment and Rationale	Outcome

EXECUTIVE SUMMARY

Page 4 Lines 2-4 (EFPIA/EBE)	Type of comment: critical It should be clarified that the guideline defines quality requirements for presentation in the Marketing Authorisation dossier and does not apply to products in clinical phases of development. "Related substances" or antibody-related substances should be removed and replaced with "antibody-related proteins". As specified in ICH Q6B, related substances are variants of the desired product and are formed during the manufacturing process and/or storage and have properties comparable to the desired product. Antibody-related proteins may be used to refer to fusion proteins, proteolytic fragments single-chain variants, and bispecific monoclonal antibodies. "This guideline lays down the product and quality control expectations for monoclonal antibodies, including generation of the cell lines for manufacturing, and other ***antibody-related proteins*** in medicinal products ***for which a marketing authorisation is being sought***. Furthermore, requirements for monoclonal antibodies and ***antibody-related proteins*** used as reagents in the production of other medicinal products are discussed. ***This Guideline is intended to facilitate the collection and submission of data to support applications for marketing authorization.***"	*In line with the Industry/BWP drafting group discussions, the scope of the document has been amended, to mainly focus on marketing authorisation, and to clearly exclude Mab used in clinical trials.* *Furthermore, as agreed during this meeting with Industry's experts, the terms "Mab-related substance" have been replaced by "Mab-related products".*
Page 4 Lines 2-3 (MEDIMMUNE)	The scope should clearly define that the requirements described are intended for a licence application. The testing required for products at earlier stages of development will depend on what is scientifically justified for example based on the link between the mechanism of action and the effector function of the molecule. Proposed new text: "This guideline lays down the quality requirements for monoclonal antibodies and related substances in medicinal products at licensure."	
Page 4 Lines 2-3 (MSD)	Clarify the applicability of this guidance to licensed products vs development product candidates: Replace "This guideline lays down the quality requirements for monoclonal antibodies and related substances in medicinal products." with, "This guideline lays down the quality requirements for monoclonal antibodies and related substances in licensed medicinal products."	

Page 4 Line 4 (WYETH)	Stage of product development / post approval life cycle not clearly specified in relation to the guideline applicability. Clarification is proposed: Added text: *This guideline pertains to the product Marketing Authorisation submission and post-approval variations, but the principles described here are applicable to all stages of product development."*	
1. INTRODUCTION		
Page no. + Line no.	**Comment and Rationale**	**Outcome**
Page 4 Lines 6-7 (MEDIMMUNE)	Replace text with (additional text underlined): "Monoclonal antibodies for human use are preparations of an immunoglobulin, or a fragment of an immunoglobulin, for example, F(ab´)2, with defined specificity, produced by a monoclonal cell line."	*Paragraph completely reworded taking into account all the comments received. See adopted text for details.*
Page 4 Lines 9-11 (MEDIMMUNE)	To bring this text up to date and be more factually accurate, additional wording for this section is required. Replace text in lines 9-11 (below): "It is currently…..in a recombinant cell culture system" with: "They can also be obtained from immortalised B lymphocytes that are cloned and expanded as continuous cell lines. Humanized monoclonal antibodies can be obtained from rDNA-engineered cell lines."	
Page 4 Lines 9-11 (EFPIA/EBE)	Type of comment: minor Production of monoclonal antibodies by hybridoma technology is state of the art, in particular for complete trifunctional (bispecific) monoclonal antibodies where there are no alternative technologies that allow obtaining intact and functional antibodies. The monoclonal antibody is expressed by an individual cell line and not by a cell culture system as the latter includes also the bioreactor and manufacturing system. Proposed rewording: "It is currently state of the art to humanise monoclonal antibodies by recombinant DNA (rDNA) technology and ***to*** express the monoclonal antibody in a recombinant cell ~~culture system~~ ***line or to obtain monoclonal antibodies by hybridoma technology***."	

Page 4 Line 18 (EFPIA/EBE)	Type of comment: major Consistent with the comment outlined above, the term "related substances" is used improperly throughout the document. This term should be reserved for the definition outlined in ICH Q6B. In some cases in the guideline, "antibody derived products" should be used or "antibody- related protein. Proposed rewording: "Finally, monoclonal antibody-related ~~substances~~ ***proteins*** are being developed, such as fusion proteins, proteolytic fragments, single-chain variable fragments and other fragments, and bispecific monoclonal antibodies (BsAb)."	
2. SCOPE		
Page no. + Line no.	**Comment and Rationale**	**Outcome**
Page 4 Line 22 (PDA)	It should be clarified that the guideline defines quality requirements for presentation in the Marketing Authorisation Application. The guideline does not apply to products in clinical development. Proposal: add line statement to read: *"The guideline defines quality requirements for presentation in the Marketing Authorisation Application. The guideline does not apply to products in clinical development."*	*In line with the Industry/BWP drafting group discussions, the scope of the document has been amended, to mainly focus on marketing authorisation. Principles of this document apply to Mab used as reagents as well as Mab-related products (to be addressed in future specific annexes, as required).* *The document now clearly excludes Mab used in clinical trials.* *The legal basis of the document has been updated to take into account the corresponding comments.* *See adopted text for details.*
Page 4 Lines 22-25 (EFPIA/EBE)	Type of comment: major The scope of this guideline needs to be clearly defined as applicable to monoclonal antibodies for which a marketing authorization is being sought and not applicable to products under development. Add the following sentences to the end of Section 2. *The guideline defines quality requirements for which a marketing authorisation has been granted or for which a marketing authorisation application will be filed. The guideline does not apply to products in clinical development."*	

Page 4 Lines 22-25 (MEDIMMUNE)	Propose to revise text to ensure the distinction between the data requirements at licensure and those for products moving through development. Replace lines 22-25 with: "In this guideline, specific requirements for registration of monoclonal antibodies for therapeutic and prophylactic use (including *ex vivo* application) and *in vivo* diagnostic use are described. In addition, requirements for monoclonal antibodies, used as reagents, especially in the purification of other pharmaceutical products are described. It is understood that the requirements should be applied on a case-by-case basis, with appropriate knowledge of the specific properties of the antibody, and that the requirements for products in development should be applied gradually as more information is generated."	
Page 4 Lines 22-25 (MSD)	Clarify the applicability of this guidance to licensed products vs development product candidates. Add line after the paragraph contained in lines 22-25: "This guideline applies to marketing applications for the production of licensed medicinal products. An appropriate subset of the requirements should be applied for the production of products for clinical use."	
Page 4, Line 27 (HAS)	The scope should be made more unequivocal. Immunoglobulin domains devoid of antigen binding site do not fit in the notion of "antibody" and the "monoclonal" nature is not applicable. Insert, following sentence 1.: The scope is restricted to proteins with antigen combining site(s). Fragments or fusion proteins including non antigen-binding regions of an immunoglobulin molecule are not covered.	
Page 4 Lines 33 (MEDIMMUNE)	Revise sentence on polyclonal antibodies. Replace text in line 33 with: "Polyclonal antibodies are outside the scope of this guideline, although some principles may apply, particularly if the polyclonal product is a mixture of monoclonal antibodies generated by rDNA technology."	

Page 4 Lines 34-36 (EFPIA/EBE) (MSD) (PDA)	Type of comment: minor Add reference to the guideline that is being replaced by this draft guideline then move these lines to Section 3 (Legal Basis) for consistency. Add this sentence to the beginning of paragraph starting at line 34 and relocate lines 34 to 36 to Section 3: ***"This guideline replaces the guideline on "Production and quality control of monoclonal antibodies", EMEA 3AB4a, July 1995."***	
Page 4 line 36 Page 9 lines 43-44 (EFPIA/EBE)	Type of comment: minor Reference is made to the Ph. Eur. monograph on "*Monoclonal Antibodies for Human use" (07/2005:2031) and (01/2005:20919) "Particulate Contamination: sub-visible particles"*. Since the Ph Eur version can change, consider deleting the version of the monograph for which this guideline is applicable. Additionally, following the outcome of this guideline, it is proposed that the monograph be updated to more currently reflect expectations for testing and control of monoclonal antibodies. Delete year of monograph (~~07/2005~~:2031) and (~~01/2005~~:20919)	

4.1. INTRODUCTORY REMARKS

Page no. + Line no.	Comment and Rationale	Outcome
4.1 Page 5 Lines 3-5 (EFPIA/EBE) (PDA) (ROCHE)	Type of comment: major "*Monoclonal antibodies are characterized by a specific structure, which is based on the immunoglobulin structure, and a clearly defined functional activity, which is primarily based on a specific binding characteristic to a ligand*" This statement is not quite correct since in many cases, functions mediated by the Fc part of the immunoglobulin structure (e.g., ADCC, CDC) significantly contribute to the function of therapeutic monoclonal antibodies. Reword last sentence:"…*which is primarily based on a specific binding characteristic to a ligand (commonly known as antigen). <u>The activity of many monoclonal antibodies is also dependant on immune effector function such as antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity</u>*	*Comments generally endorsed, however, since the "introductory remarks" proposed in the initial draft were mainly descriptive of current technologies which will most likely evolve, and did not bring much recommendation, most of these remarks were deleted from the document. Where relevant, these comments were taken into consideration in drafting other sections.*

4.1 Page 5 Lines 7-8 (EFPIA/EBE) (PDA) (ROCHE)	Type of comment: major "*Based on their structure, monoclonal antibodies can be nonhuman, chimeric/humanized or human antibodies*" This statement is too simple. The list is not complete because monoclonal antibodies are not covered where immunogenicity has been decreased e.g. by using algorithms to identify T cell epitopes via *in silico* screening of the amino acid sequence of therapeutic proteins, followed by replacement of these T-cell binding sequences, in order to diminish the immunogenicity of these products. Additionally, monoclonal antibodies modified either in their amino acid sequence or glycosylation with the aim to enhance, or reduce, immune effector functions have recently become available. Both these classes of products are in clinical studies now, so that it can be expected that products of this type will be submitted for licensing in near future. Thus, they should be covered explicitly by the guideline. Add sentence: *"Furthermore, monoclonal antibodies can be modified concerning primary structure as well as glycosylation in order to modify their immunogenicity or immune effector functions."*	
Page 5 Line 13 (HAS)	Besides clinical adverse reactions the therapeutic effect can also be modified and cause unwanted side effects. *Insert the following extension:* …adverse reactions and/or modify the therapeutic potential that may cause unwanted side effects.	

4.1 Page 5 Lines 13-14 (EFPIA/EBE)	Type of comment: major Clarification is required when referencing "sequence" to specify whether it refers to the protein or genetic sequence. EFPIA/EBE agrees that the anti-antibody response should be considered. Nevertheless the advantages of non-humanised antibodies should be considered. In particular a non-humanised Fc region can allow a selective activation of the immune response. On the other hand non humanisation of the constant regions allows correct species-specific pairing of light and heavy chains and this is of paramount importance for the production of some monoclonal antibodies as the intact trifunctional (bispecific) monoclonal antibodies. Furthermore, even for proteins with a full human sequence an anti-protein response is known. Change as follows: This may result in clinical adverse reactions. As a consequence, ~~the use of~~ ***when using*** antibodies ***generated from a*** ~~a full~~ non-human ***genetic*** sequence, ***anti-antibody response should be determined.***	

4.1 Page 5 Line 15 (EFPIA/EBE) (PDA) (ROCHE)	Type of comment: critical *"Human monoclonal antibodies are antibodies of entirely human sequence"*: This statement is not scientifically correct because even "human" monoclonal antibodies used as therapeutic proteins, depending on the way they are obtained (cf. chapter 4.2.3), may contain sequences which are product of in-vitro (e.g., phage display) or in-animal (e.g., transgenic technologies) selection and thus have to be considered foreign to the human body. Reword the sentence accordingly: *"Human monoclonal antibodies are antibodies <u>entirely derived from human germline immunoglobulin</u> sequences."* Regarding the definition of human monoclonal antibody, the artificial amino acid replacement and the use of artificially synthesized sequence should be considered. We regard that a human monoclonal antibody is an antibody entirely derived from human germline immunoglobulin sequences both in variable regions and constant regions. Artificial replacement of certain amino acids may be performed in order to enhance and/or reduce its affinity and/or other properties. However, such an artificially modified antibody is no longer a human antibody, if the categorization is primarily based on the consideration of the potential risk of immunogenicity. Some antibodies are generated from a certain synthetic antibody library using human germline frameworks, but this type of antibody is also not in the scope of human monoclonal antibody for the same reason. However, in a very real sense, the distinction between chimeric, humanized and human antibodies is meaningless from the viewpoint of immunogenicity (please see our general comment above). Add a sentence after "*<u>entirely derived from human germline immunoglobulin</u> sequences."* (see above): *"If a monoclonal antibody includes any artificial amino acid replacement and/or artificially synthesized sequence which are not found in the human germline immunoglobulin sequences, such a monoclonal antibody is not a human monoclonal antibody and should be categorized as non-human, chimeric or humanized monoclonal antibody."*	

4.1 Page 5 Line 15 (MEDIMMUNE)	It should be made clear that it is the amino acid sequence that is being referred to here. The carbohydrate structures may be non human. It is proposed to amend the text to: "Human monoclonal antibodies are antibodies of entirely human amino acid sequence."	
4.1 Page 5 Line 15 (ROCHE)	Regarding the definition of human monoclonal antibody, the artificial amino acid replacement and the use of artificially synthesized sequence should be considered. We regard that a human monoclonal antibody is an antibody entirely derived from human germline immunoglobulin sequences both in variable regions and constant regions. Artificial replacement of certain amino acids may be performed in order to enhance and/or reduce its affinity and/or other properties. However, such an artificially modified antibody is no longer a human antibody, if the categorization is primarily based on the consideration of the potential risk of immunogenicity. Some antibodies are generated from a certain synthetic antibody library using human germline frameworks, but this type of antibody is also not in the scope of human monoclonal antibody for the same reason. However, in a very real sense, the distinction between chimeric, humanized and human antibodies is meaningless from the viewpoint of immunogenicity (please see our general comment above). Add a sentence after "*… of entirely human sequence*"; *"If a monoclonal antibody includes any artificial amino acid replacement and/or artificially synthesized sequence which are not found in the human germline immunoglobulin sequences, such a monoclonal antibody is not a human monoclonal antibody and should be categorized as non-human, chimeric or humanized monoclonal antibody."*	

4.1 Page 5 Lines 16-20 (MEDIMMUNE)	To bring this text up to date and be more factually accurate, additional wording for this section is required. Replace text in lines 16-20 with: "rDNA engineered antibodies include: Recombinant human monoclonal antibodies: the variable heavy and light chain domain of a human antibody are combined with the constant region of a human antibody. Chimeric monoclonal antibodies: the variable heavy and light chain domains of a human antibody are replaced by those of a non-human species, which possess the desired antigen specificity Humanized monoclonal antibodies: the three short hypervariable sequences (the complimentary determining regions) of non-human variable domains for each chain are engineered into variable domain framework of a human antibody; other sequence changes may be made to improve antigen binding.	
4.1 Page 5 Line 18-20 (EFPIA/EBE) (PDA) (ROCHE)	Type of comment: major Reference comment in section 4.1, line 7-8 Monoclonal antibodies where immunogenicity has been decreased e.g. by using algorithms to identify T cell epitopes via *in silico* screening of the amino acid sequence of therapeutic proteins, followed by replacement of these T-cell binding sequences ("T-cell epitope depleted" antibodies) are not mentioned and should be included in the scope of this document. Clarification is required when referencing the term "sequence" whether it refers to the protein or genetic sequence. Reword and add sentence at end of line 20: Humanised monoclonal antibodies are antibodies in which the three short hypervariable regions (the complementarity determining regions or CDR's) of non-human variable domains ~~for each chain are engineered into~~ of each chain ~~are engineered into~~ ***have been grafted onto the framework regions of the*** variable domains of a human antibody. ***Other approaches to reduce immunogenicity of animal monoclonal antibodies by in silico or in vitro techniques are emerging.***	

4.1 Page 5 Lines 21-24 (MEDIMMUNE)	The text should include reference to conjugated fragments i.e. PEGylated Fabs. It is proposed to amend the text to: "In addition to the full-length, non-modified monoclonal antibodies, there are other monoclonal antibody related substances that fall within the scope of this Guideline, such as antibody fragments (including scFv), fusion proteins, bispecific antibodies, conjugated monoclonal antibodies, conjugated antibody fragments or radiolabelled monoclonal antibodies."	
4.1 Page 5 Line 24 (EFPIA/EBE)	Type of comment: minor Consider mentioning only "labelled" antibodies, since label may be different from radioactive (fluorescent, gold-particles, etc. …) Type "labelled antibodies" or "(radio-)labelled antibodies"	
4.1 Page 5 Lines 25-27 (EFPIA/EBE)	Type of comment: minor This introduction is not consistent with the actual sections presented below it. Change line 25 sentence to read as follows: This guideline covers ***principles and general requirements of the cell line generation,*** production, characterisation, ~~specifications, and formulation, comparability,~~ and specification to be used as, or in the production of, human medicinal products.	
4.1 Page 5 Lines 25-31 (MEDIMMUNE)	The guideline should clarify that the requirements are different for different types of Mabs, and that the amount and type of information should be related to the mechanism of action and the correlation to effector function. The introduction should again point out that what is described here applies for registration and that the level of information required will be different for different stages of development. New text to come after the end of line 31: "The extent of these recommendations will be based on their specific properties. For example, requirements relating to the Fc part of a monoclonal antibody (including effector functions) are not relevant for Fab fragments or for monoclonal antibodies that are demonstrated to lack effector function."	

4.1 Page 5 Lines 34-40 (EFPIA/EBE) (MSD) (ROCHE)	Type of comment: critical As this guideline lays down quality requirements, a discussion on pre-clinical investigation in animals is not appropriate for this guideline. Please note that the wording as stated, "it should be noted that the use of these data is limited by the fact that quality characteristics of the clinical and homologous monoclonal antibody are different and that it is therefore difficult to extrapolate the data obtained" largely invalidates the use of homologous antibodies and therefore conflicts with regulatory guidance in ICH S6. It should be clarified that the use of surrogate molecules can be valuable in pharmacological studies provided that the surrogate is shown to be representative of the antibody intended for clinical use. The increase in mAb development and the high species specificity has led to the well-founded concern by the pharmaceutical industry that the number of OLD World primates for safety and efficacy testing might increase and that data from chimpanzee, a protected species, might be requested. Alternatively, genetically altered rodents expressing the appropriate human transgene and surrogate antibodies have been used successfully in the development for preclinical efficacy and safety assessments. The presence of the human transgene allows the clinical antibody to be tested on the human target antigen. For example, Raptiva a primatized (monkey and human) anti CD4 mAb only binds to human and chimpanzee CD4. Although the chimpanzee was used for limited safety studies to support first time in man studies, a humanized transgenic mouse expressing human CD4 was used extensively for PK/PD studies, single-and repeat-dose toxicity studies, host defence and also to address the safety concern of anti-CD4 immunosuppression as an adverse side effect. The PK/PD studies in the humanized mouse showed that Raptiva produced the expected decrease in the number of CD4 expressing T-cells and reduced the CD4 on the T cell surface. Similar results were obtained in the clinical studies supporting the relevance of the transgenic model in preclinical safety studies.	*All text relating to homologous Mab and non clinical studies has been removed from the document.*

	Proposed rewording: "For preclinical investigations in animals, homologous monoclonal antibodies (monoclonal antibodies which recognise the same ~~epitope, but have a different structure, for example murine or simian instead of human IgG~~ ***target in the relevant preclinical species***) are sometimes developed. Although using homologous monoclonal antibodies may be the only feasible way to perform pharmacological studies in animals, and the results may have scientific value, it should be noted that ***use*** ~~the extrapolation~~ of these data ***to extrapolate the PK/PD characteristics of the homologous clinical antibody must be justified.*** ~~by differences between the characteristics of the clinical and homologous monoclonal antibody. should be noted that the use of these data is limited by the fact that quality characteristics of the clinical and homologous antibody are different in most situations, and that it is therefore difficult to extrapolate the data obtained.~~ ***The suitability of the data extrapolation of such studies supporting certain conclusion will be reviewed on a case-by-case basis. As an alternative, transgenic animal models expressing the human target antigen might be used."***	
4.1 Page 5 Lines 34-40 (MEDIMMUNE)	The advice on use of homologous reagents should be consistent with other guidance e.g. First in Human Clinical Trial Guidance (EMEA/CHMP/SWP/28367/07). Replace with: "For preclinical investigations in animals, the use of homologous proteins (monoclonal antibodies which recognize the same epitope but have a different structure, for example murine or simian instead of human IgG or fusion proteins) may be the only choice." Although using homologous proteins may be the only feasible way to perform pharmacological and toxicological studies in animals… are different, and this should be taken into account when extrapolating the data obtained."	

4.1 Page 5 Lines 34-40 (PDA)	We propose replacement of the first sentence referring to preclinical development with a general statement that encourages innovative approaches yet allows companies to protect currently used technology. Replace paragraph starting on line 34 with: "*Several approaches are currently available during product development and firms should review their strategies with the regulatory authorities in order to determine the relevance and applicability of that data."* Furthermore, we suggest qualification of the statement that use of data generated using homologous antibodies is of limited value and identification of arena where data may or may not be applicable (lines 36-40): *"When homologous monoclonal antibodies (monoclonal antibodies which recognise the same target in the relevant preclinical species) are used to collect scientific data, the use of the data must be adequately justified and take into consideration the degree of comparability to the clinical candidate with respect to attributes such as production process, range of impurities/contaminants, pharmacokinetics and pharmacological mechanism. The homologous monoclonal antibody does need to be well characterized".*	

4.2. GENERATION OF THE MONOCLONAL CELL LINE

Page no. + Line no.	**Comment and Rationale**	**Outcome**
4.2 Page 5 Lines 42-43 (EFPIA/EBE)	Type of comment: minor Also production of monoclonal antibodies by hybridoma technology is state of the art, in particular for complete trifunctional (bispecific) monoclonal antibodies where there are no alternative technologies that allow obtaining intact and functional antibodies. Delete sentence.	*Most of these comments are endorsed, and were taken into account in the adopted version of the document.* *In summary, no technology is a priori banned, however the selected approach should be appropriately justified and documented. Special warnings are made for approaches that may present higher risk with regards to adventitious agents.*
4.2.1 Page 6 Line 3 (MEDIMMUNE)	Change the order of sections 4.2.1 and 4.2.2. Start with "Recombinant DNA technology in antibody production", then describe hybridoma-derived antibodies, as rDNA technology is 'state of the art'.	

4.2.1 Page 6 Lines 4-23 (PDA) (ROCHE)	Regarding the statements of EBV and vCJD, throughout the guideline very specific references are made, a more generalized approach should be used to address the principal and the approaches to be used. Similarly it appears that most references to Mab are IgG specific and a more general approach would be more useful, or discuss the differences for IgG, IgM, IgE, fragments and fusion proteins. Suggested rewording of the section to address this. Line 17: The parental cell line should be documented also with respect to raw materials to which it has been exposed (obviously focused on those of human and/or animal origin). Suggest rewording the section to read: *4.2.1 Hybridoma* *Hybridomas are cell lines created through the fusion of murine B lymphocytes with myeloma cells to achieve immortalization. These are acceptable systems for the production of monoclonal antibodies; however the choice of the system should take into consideration the antigenicity issues related to non-human antibodies, as well as viral safety issues related to cell lines. The method of transformation needs to be assessed for potential safety concerns.* *The use of continuous human B-lymphocyte as parental cell lines raises specific concerns regarding the transmission of infectious agents and pathogens. The choice of human cell lines and the method of transformation should be cautiously considered and appropriately justified. The immortalization of a human or non-human B-lymphocyte through cell fusion or transformation may be necessary to obtain a stable and continuous monoclonal cell line. Source cells include lymphocytes, myeloma cells, feeder cells and host cells for the expression of the protein. The origin and characteristics of the parental hybridoma or recombinant cell line should be documented, including information regarding the health history of the donors, the fusion partner used, and raw materials of animal/ human origin to which it has been exposed.* *The use of ascites as a production system for hybridoma-derived monoclonal antibodies is discouraged in view of viral safety issues and in light of the principles of Directive 86/609/EC, which seeks to reduce, refine and replace the use of animals for these purposes."*	

4.2.1 Page 6 Line 18 (HAS)	Sentence "Hybridoma cell lines are acceptable…." should be deleted. The therapeutic use of murine hybridoma derived, as well as the virus transformed human B-cell derived monoclonals are discouraged in the previous items. In most instances monoclonals are expressed in a host cell line (CHO or Sp2) and produced by fermentation. A wording corresponding to the above consideration is proposed. Replace sentence "Hybridoma cell lines…" by: Appropriate identification and characterization of the cell line used for the expression of a therapeutic monoclonal antibody should be provided. The viral safety issues related to the host cell line should be addressed.	
4.2.2 Page 6 Lines 25-31 (PDA)	More detail should be provided regarding the requirements and documentation for the recombinant expression systems. The statement on specific procedures… "do not need to be described in detail." is confusing; rather the section should outline what should be documented and detailed with regard to transformation, amplification procedures Statement to read: *''4.2.2. Recombinant DNA technology in antibody production:* *A description of the expression system used for the production of antibodies including the expression constructs and characterization of the rDNA expression vector and parental cell line should be provided.* *When one or more specific procedures are performed prior to the isolation of the gene of interest, such as cell fusion, viral transformation, gene library of phage display screening, these procedures do not need to be described in detail, however appropriate information regarding the source and cloning of genes should be provided.''*	
4.2.2 Page 6 Line 27 (EFPIA/EBE)	Based on the general nature of several statements within this guideline, It would be helpful to include reference to the applicable ICH document for more specific detailed requirements. At the end of line 27 include the following sentence: ***"Refer to ICH Q5B: Quality of Biotechnological Products : Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products, for more detailed requirements."***	

4.2.2 Page 6 Lines 28-30 (MEDIMMUNE)	*"…these preceding procedures do not need to be described in great detail."* Clarification is sought as to the level of information that would suffice.	
4.2.2 Page 6 Line 29 (EFPIA/EBE) (PDA) (ROCHE)	Typo. Correct *"…to be used to <u>obtain</u> the …"*	
Page 6 4.2.2 4.2.3 Lines 29-34 (EFPIA/EBE)	Section 4.2.2 and 4.2.3 provide a general list of common technologies used to generate the antibody. It is requested that some clarification and redundancies be eliminated or re-worked. For example, transgenic mice make human antibodies but don't make human B-lymphocytes. There are huSCID mouse models in which human hematopoietic stem cells are transplanted into immune compromised mice that can make human B-lymphocytes. Phage display also does not make monoclonal cell lines. These are made using recombinant technology as described in 4.2.2. Not mentioned were direct isolation of human B-cells and single cell cloning of the heavy and light chains without immortalization, and additionally, other technologies include making hybridomas with human B-cells.	
4.2.3 Page 6 Line 33-34 (EFPIA/EBE) (PDA) (ROCHE)	Type of comment: minor Suggested wording change for correct of use of creating the antibody rather than for generation of the cell line expressing the antibody: "Other technologies, such as development of transgenic mice to generate human B-lymphocytes or phage display, may be used to generate the ~~monoclonal cell line~~ ***human immunoglobulin***."	

4.3. PRODUCTION OF MONOCLONAL ANTIBODIES		
Page no. + Line no.	**Comment and Rationale**	**Outcome**

4.3.1 Page 6 Line 40 (EFPIA/EBE)	Type of comment: minor Consistent with ICH Q7A, validation should be performed for those operations determined to be critical to the quality and purity of the drug substance. Use of the word, "fully" is ambiguous and can lead to confusion. Further clarification is provided regarding the expectation for completion of validation activities to support marketing authorization. Proposed rewording: "The ~~All parts of the~~ drug substance manufacturing process (cell culture, purification, etc.) should be ~~fully~~ validated ***for commercial licensure and/ or prior to distribution of a licensed product in accordance with ICH Q7A.***"	*The text regarding manufacturing process has been completely revised, and mainly states that the process should be appropriately described and validated.* *Many of the comments made on the points to be considered in process validation were not included in the adopted guideline as they are not specific to Mab, and are applicable to all biotech products. Such elements should be considered in the drafting of ICH Q11 document.*
4.3.1 Page 6 Lines 40-41 (MEDIMMUNE)	*"All parts of the drug substance manufacturing process….should be fully validated."* It is assumed that the term "fully validated" relates to the process in Phase 3 or later in development. It is often the case that the final process is not established until Phase 3 or just prior to licensure. A phased approach to validation is also assumed, with increasing validation as the product and process move through later stages of development. In addition, advice should be given on expectations for validation at various stages of development, dependent on the product characteristics, mechanism of action and therapeutic use i.e. for life threatening versus non-life threatening conditions. It is proposed to amend the text to: "Using a phased-validation approach, appropriate for the product, all parts of the drug substance manufacturing process….should be fully validated by the time of MAA. Reference is made to the Ph. Eur. Monograph on "*Monoclonal antibodies for human use* (07/2005: 2031)."	

4.3.1 Page 6 lines 40-43 Page 7 line 1 (PDA) (ROCHE)	**PDA:** The drug substance manufacturing process should be qualified or validated consistent with the stage of product and clinical development, focusing specifically on those characteristics which impact the final product specifications and clinical efficacy. **ROCHE:** This document states that "*All parts of the drug substance manufacturing process … should be fully validated*." Recommend deleting reference to "all" and clarifying that most aspects of drug substance manufacturing processes need not be validated prior to commercial manufacturing. **PDA/ROCHE:** It is unclear what is meant with "consistency of production with respect to heterogeneity". Later (chapter 4.4.1.2) it is correctly stated that a full identification of all minor species will not be possible; on the other hand, "consistency" has to be ensured not only with respect to heterogeneity, but regarding all aspects of the manufacturing process. Delete "*with respect to heterogeneity*". The section should be reworded as follows: ''*The drug substance manufacturing process (cell culture, purification, etc) should be fully validated at the time of submission of a Marketing Authorisation Application.* *While establishing the process and its capabilities, attention should be focused on ensuring that the product quality attributes are consistently met both for critical in-process steps and for the drug substance release. These include batch to batch variations in heterogeneity, purification capability and process related impurities.* *During development, the stability of the cell-line and the process conditions are determined and then transferred to the production scale. The capabilities of the production where also, particular attention should be paid to genetic stability, scale should be confirmed and the product quality attributes verified.*	

	Typically the review should include: *– Consistency of product attributes for example, potency specific activity.* *– Consistency in the removal of product and process-related impurities [for example host cell protein (HCP), DNA, purification related substances such as protein A]*.* *Attention should be paid to ensure that the drug substance when formulated to drug product is capable of meeting the requirements of Ph. Eur. for parenteral preparations."* *: ROCHE proposal: "….(*for example host cell protein (HCP) and DNA, protein A, antibiotics, cell culture components) <u>and viruses</u>;"*	
4.3.1 Page 6 Line 42 (EFPIA/EBE)	Type of comment: major It is unclear what is meant by "consistency of production with respect to heterogeneity". Later, in (chapter 4.4.1.2), it is correctly stated that a full identification of all minor species will not be possible; on the other hand, "consistency" has to be ensured not only with respect to heterogeneity, but regarding all aspects of the manufacturing process. Suggested re-wording: -consistency of production with respect to ***product quality, specifically critical quality attributes*** ~~heterogeneity~~ ***and critical process controls***	
4.3.1 Page 6 Line 42 (HAS)	Heterogeneity is not the only aspect to evaluate when assessing production consistency. Replace by "consistency of production with respect of identity, purity and structural heterogeneity;"	

4.3.1 Page 6 Line 43 (EFPIA/EBE)	Type of comment: major Section 4.3.1, line 43 states the process should be "validated for adequate removal of process- and product-related impurities". However, If controlled as part of the release specification, then the process for removal of these impurities should not need to be validated. The scope of required validation is too broad. Validation of all these aspects should afford the sponsor some flexibility in the form of reduced specification release tests. Add to the end of this section, page 7, line 15: "***Validation to remove process- or product-related impurities can be used to justify exclusion of routine release testing for these impurities.***"	
4.3.1 Page 7 Line 1 (EFPIA/EBE)	Type of comment: major Cell culture cultivation media are highly complex and contain ingredients at or below the limits of detection using currently available methods for analysis. Additionally, it can be justified that not all media components should be validated for removal based on their mass and charge it is obvious that some components are inherently removed from the process; however it is understood that an assessment is conducted and any unknown, active or toxic components should be validated for removal. Suggested rewording: "…and DNA, protein A, antibiotics, ***relevant*** cell culture components)" Add the following statement at the end of page 7, line 1. ***"When appropriate, the removal of cell culture components should be demonstrated or the residual level shall be justified based on the expected extent of removal by the purification process, dosing scheme, and a toxicology assessment."***	
4.3.1 Page 7 Line 1 (MSD)	Clarify the "cell culture components" in the removal of process impurities discussion since cultivation media are highly complex and contain ingredients at or below the limits of detection of currently available assays. Add the following statement at the beginning of page 7, line 1: "When appropriate, the removal of cell culture components can be justified by factoring the extent of dilution achieved during the purification steps."	

4.3.1 Page 7 Lines 2-7 (MSD)	Regarding the "compliance with requirements for the bacterial endotoxins test". A reference should be given to the endotoxins requirements.	
4.3.1 Page 7 Lines 4-7 (PDA) (ROCHE)	After "non-endotoxin contaminants," seems to be phrased very generally. Does this mean any amount of any contaminant? It's not clear what would trigger such testing and, thus, this is a risk of becoming a standard, yet ill-defined, expectation. Would suggest rewording. In addition to endotoxin testing there is the requirement for a monocyte activation test if there is the likelihood of the presence of non-endotoxin contaminants. The monocyte activation test is redundant to other well-established tests like testing for bioburden and endotoxins. In addition there is up to now no monograph for the monocyte activation test in place which also illustrates that this is an unusual test. As there is no substantial added value of this test we would recommend deleting the reference to this test. Reword this sentence: *"…indicates the likelihood of the presence of non-endotoxin, pro-inflammatory contaminants, such as peptidoglycan, additional testing should be considered."*	
4.3.1 Page 7 Line 4 (HAS)	For sake of clarity "non-endotoxin contaminants" should be specified. Proposed wording: "… non-endotoxin inflammatory contaminants".	
4.3.1 Page 7 Line 6 (HAS)	We propose rewording the last sentence of the paragraph. "Such a test would also be expected to identify unwanted activation of the innate immune system including the release of pro-inflammatory mediators."	

4.3.1 Page 7 Line 8-9 (EFPIA/EBE)	Type of comment: major This statement regarding development and validation, in particular genetic stability, optimal and validated time for fermentation and harvests is somewhat ambiguous and inconsistent with current regulatory guidance. For example, genetic stability is typically conducted on 1 lot and testing is limited to viral contaminants. It is proposed that since validation should be performed for those operations determined to be critical to the quality and purity of the drug substance, that the prescriptive nature of these details are not necessary for this discussion. Delete lines 8-9	
4.3.1 Page 7 Lines 8-9 (MEDIMMUNE)	As noted above, the level of validation and the requirement for certain information should be linked to the phase of development. Process optimisation will not normally be completed until Phase 3 (or post Phase 3), and hence optimal harvest points will not be fully defined until the process is finalised. Replace text with: "During development, particular attention should be paid to genetic stability. Data on process performance, optimal conditions for fermentation and harvest (yield, product quality) etc and consistency should be generated as more experience is gained during process validation."	
4.3.1 Page 7 Lines 8-9 (ROCHE)	Strike the word "optimal" from this sentence. Optimization (or not) of the fermentation and harvest timing is up to the manufacturer and should not be subject to regulatory requirements. Reword this sentence: *"During development and validation, particular attention should be paid to genetic stability, validated time for fermentation and harvest (yield, product quality), etc."*	

4.3.1 Page 7 Lines 8-15 (PDA)	The section 4.3.1 general considerations relates mostly to other documents and in particular to GMP Volume 4 of the Eudralex Part 2 and several Annexes. As these documents specify the requirements for validation, manufacturing consistency, production controls etc. delete lines 8-15 as this is specific to one type of purification and to one step in purification only. Refer to Eudralex Volume 3, Biotechnology guidelines for specific safety requirements for materials of biological origin used in the process and production.	
4.3.1 Page 7 Line 2-7 (EFPIA/EBE)	*"-compliance with the……cytokines".* The statement: "a monocyte activation test for pro-inflammatory and pyrogenic contaminants might provide valuable information" is ambiguous and could be interpreted to mean that such an assay would be expected to be performed routinely. The monocyte activation test suffers a number of limitations as regards to robustness and practicality for routine use and is therefore not considered appropriate as an in-process control and/or specification test. Additionally, the monocyte activation test is redundant to other well established and recognized analytical methods for determining bioburden and bacterial endotoxins. The non-specific nature of this testing challenges the value, and the relatively high chance of false positives is a problem. Proposed rewording: "-compliance with the requirements for the bacterial endotoxins test ***(or rabbit pyrogen test)***. In addition, where the manufacturing process itself or the data from the analysis of product- and process-related impurities indicates the likelihood of the presence of non-endotoxin, ***pyrogenic*** contaminants, such as peptidoglycan, ***additional testing should be considered for characterization purposes.***"	*Agreed, section moved to characterisation and text amended.*
4.3.1 Page 7 Lines 10-15 (PDA)	Suggest to delete the reference to Protein A, as this approach is not "an almost universal approach" to purification.	*Agreed, text reworded.*

4.3.1 Page 7 Lines 11-15 (EFPIA/EBE) (MSD)	Type of comment: major The wording in this section can lead to misinterpretation. Suggested changes reflect the concern that use of human IgG anywhere associated with the manufacturing process should take into consideration the regulations and requirements to ensure safety from contaminants in the product manufactured. Modify lines 11-15 to read as follows: "***Protein A used as the affinity ligand in the production of protein A resin is obtained from S. aureus or from recombinant sources. In those cases where the purification of the protein A ligand employs the use of human IgG that is purified from blood, the quality of the human IgG needs to be appropriately documented for the intended use..***...".	
4.3.1 Page 7 Line 14 (EFPIA/EBE)	Type of comment: minor Avoid reference containing distinct year (01/2007:0338) but rather use generic reference.	

4.3.2 Pages 7-8 (PDA) (ROCHE)	In general, Section 4.3.2 on Platform Manufacturing is confusing. Consider rewriting, and setting clear guidance on how data from platform manufacturing can be used for registration of different monoclonal antibodies. This might be presented in the context that the entire manufacturing sequence is an integrated process which must be considered at each step in the process as well as in its entirety. The section should be more advisory describing how platform manufacturing could be used and what data is required to support each product submission when such an approach is applied. Data to support each product submission should be provided on a product by product basis to support the product manufacturing independent of whether or not a common platform approach or unique process is used. The full data package should be provided even if there is generic data common to more than one product. A company should be able to choose whether or not it uses a platform approach on unit operations or a process. The sentence of acceptability of platform manufacturing approach for process development should be deleted since it is the choice of the company to choose the manufacturing strategy. Please refer to the following specific comments on chapter 4.3.2, to clarify to the reader how a manufacturing platform can be applied and used.	*The section on platform manufacturing has been significantly amended to take into account the comments received. The adopted document introduces the possibility of reducing validation data to be submitted, when supported by data demonstrated to be relevant for the final commercial process.* *Following the Industry/BWP meeting, the text was clarified to include careful recommendations when using platform manufacturing approach in virus validation studies.* *See adopted guideline for details.*

4.3.2 Page 7 Lines 17-22 (EFPIA/EBE)	This paragraph is unclear as written and should be revised to provide clarification to the definition of "platform manufacturing" and to include relevance to the advantages of this concept. Proposed rewording: The structural and physiochemical characteristics of monoclonal antibodies are ~~now~~ well understood. ~~and together with many years of antibody process development and production in industry, a concept whereby different monoclonal antibodies are manufactured using identical, pre-defined purification process has evolved.~~ ***In conjunction with many years of antibody process development and production in industry,*** ~~a concept whereby~~ ***different monoclonal antibodies are manufactured using similar manufacturing processes using a pre-defined host cell, cell culture, and/or purification process.*** ~~has evolved~~. ***This concept is termed "platform manufacturing" and is unique for each manufacturer and shall be described as such.*** ***A "platform manufacturing" approach can reduce regulatory burden by facilitating the extrapolation of data (such as process validation, viral validation, resin re-use, and definition of CPPs) across products utilizing the platform, if appropriately justified.***	
4.3.2 Page 7 Line 19 (MEDIMMUNE)	Platform processes, although similar, are generally not identical. Revise text: "….whereby different monoclonal antibodies are manufactured using <u>similar</u>, pre-defined purification……".	
4.3.2 Page 7 Line 21 (PDA) (ROCHE)	Word "*identical*" is misleading, please rephrase. Replace with "*comparable*".	

4.3.2 Page 7 Lines 23-26 (EFPIA/EBE)	Type of comment: major This paragraph is unclear as written and should be revised to provide clarification to the concept as it applies to stages of development. Suggested re-write of lines 23-26: ***"A "platform manufacturing" approach can be utilized in both process development and commercial manufacturing processes, because it is not necessary to develop each new manufacturing process de novo. Instead it is based on previously developed in-house knowledge and may also be appropriate to make efficient use of available or shared equipment during development and for commercial manufacturing."***	
4.3.2 Page 7 Lines 27-29 (EFPIA/EBE)	Type of comment: major Suggested re-wording to provide clarity regarding verification required for each new molecule used in a platform process. Suggested rewording: "However, each producer cell lines (even if derived from the same parent cell line and a similar genetic construct) ~~is likely to~~ ***may have*** different characteristics. Therefore, any ***significant*** product-specific process should be duly optimised and validated in its own right. ~~This also applies to process changes~~ ***unless the properties fall within an established design space for the platform and process verification and/or evaluation has been demonstrated.***"	
4.3.2 Page 7 Lines 27-29 (PDA) (ROCHE)	Strike the word "*optimised*" from this sentence. Additionally, the wording in this paragraph (lines 27-29) should be improved. Suggest rewording. *However, each producer cell line (even if it derived from the same parent cell line and a similar genetic construct is used) may have different characteristics. Therefore, any product-specific process should be duly validated in its own right. The manufacturer, however, may rely on process characterization and/or validation data obtained with other products manufactured using the same platform cell culture process if the data relevance is justified. This applies to both new processes and process changes."*	

4.3.2 Page 7 Line 30 (ROCHE)	Replace “will never” with “may not” or “will probably not”. Reword this sentence: *“The “platform manufacturing” process <u>may not</u> be identical for each monoclonal antibody…”*	
4.3.2 Page 7 Lines 30-31 (PDA)	Replace concept of identical with statement on the variability as “*The “platform manufacturing” process may vary for each monoclonal antibody depending on the unit operation and operational control.”*	
4.3.2 Page 7 Line 31 (HAS)	The first sentence of this item is not clear enough, we propose a rewording: “…for each monoclonal antibody and specific requirements have to be considered.”	
4.3.2 Page 7 Line 31 (MSD)	Needs clarity on the intent of the phrase "interference by the product....". Modify line 31 to read "… interference in the performance of the platform process by each new product…"	

4.3.2 Page 7 Lines 30-38 (EFPIA/EBE)	Type of comment: major Clarification is required to fully understand the implications of 'interference by product'. Additionally, although each product may be unique, platform manufacturing processes may only vary slightly and reliance on supporting data may be important to establish design space for a given product and process, as well as for viral safety evaluation. Additionally, as a company's knowledge of the use of platforms develops, the ability to utilise "prior knowledge" will increase over time. It is the responsibility of the manufacturer to justify what data he has to support his claim that the process as applied to the particular product has acceptable viral clearance. *Proposed re-write of this section:* ***The manufacturer, however, may rely on process characterization and/or validation data obtained with other products manufactured using the same platform process if the data relevance is justified. This applies to both new processes and process changes.*** ***The "platform manufacturing" process may not be comparable for each monoclonal antibody and interference related to the performance of the platform by each new product cannot be excluded beforehand.*** ***Therefore each process should be separately evaluated for its ability to clear/inactivate viruses. However, for a new product, the manufacturer may rely on viral clearance/inactivation data obtained with other products manufactured with the same "platform manufacturing" process, as justified. Extrapolation of viral validation studies must be supported by a thorough evaluation of the process parameters that affect virus reduction, a demonstration of similar biochemical properties of the products in the platform, a critical analysis of the manufacturing step, and confidence that the manufacturing step provides similar capacity to inactivate/remove potential virus contaminants.*** ***With respect to data relating to cleaning effectiveness and regeneration procedures, data may be extrapolated across from the same "platform manufacturing" processes provided this can be fully justified.***	

4.3.2 Page 7 Lines 31-38 (PDA) (ROCHE)	The comments should be kept general. The sentences in this paragraph are confusing and raise questions as follows: 1) If the process is identical, what data from the 'new product' would be necessary? Would virus particle counts on the harvest be adequate to show that the number of particles in the harvest of the new product is within the validated range? If not, what else would be needed? 2) If only one parameter of one step in the platform process is changed for the new product, is revalidation of that step sufficient? 3) If validation of the platform process includes ranges of conditions for each step and all new products are purified within those ranges, does additional validation need to be done for a new produced (except #1 above)? It would be helpful to have examples of which data can be regarded as supportive: thereby permitting a reduced program to be performed. In principle it would be helpful to harmonize the requirements with the relevant guidelines for virus safety and to include the respective references. Suggest the following text: *The "platform manufacturing" process may vary for each monoclonal antibody depending on the unit operation and operational control. Data from a 'platform manufacturing' process may be considered supportive but the manufacturer will need to justify the relevance of the data used. Therefore, data to support each product submission should be provided on a product by product basis whether or not a common 'platform manufacturing' or if a unique approach is used.*	
4.3.2 Page 7 Line 34 (MSD)	As written this guidance suggests the same level of viral clearance validation is needed for clinical supplies as for commercial product. A reasonable interpretation of the needs would be served by inserting a phrase as follows: After the phrase "Such data may be considered supportive but…" insert "…in the license application…". This would need to be reconciled with the Draft "Guideline on virus safety evaluation of biotechnological investigational medicinal products" (EMEA/CHMP/BWP/398498.2005_corr).	

4.3.2 Page 7 Lines 34-36 (ROCHE)	What is involved in demonstrating that virus validation data for a new product are comparable to data obtained from other products? It sounds like you'd have to re-validate whether you stayed within the previously validated conditions or not. Recommend ending the sentence after "justify the relevance of the data.". Reword this sentence: "*Such data may be considered supportive but the manufacturer will need to justify the relevance of the data."*	
4.3.2 Page 7 Lines 39-43 (PDA) (ROCHE)	This paragraph is very unclear. It is not clear if they are discussing the ability to implement global changes to approved processes or the need to revisit the ability to apply modular concepts to new products prior to licensure when the "Platform changes": Suggest rewording: *"If a change is made to the platform process which will be implemented globally on several marketed products which had been previously validated using a combination of platform knowledge and product specific confirmatory data, revalidation of the process performance related to the proposed change should be reconfirmed for each product unless it can be justified that results supporting the change can be extrapolated across the platform. Use of family, modular and bracketed approaches to validation is encouraged."*	

4.3.2 Page 7 Lines 39-43 (EFPIA/EBE)	Type of comment: major The guidance refers to partial validation and re-validation as a means of confirming that any changes implemented to a platform process do not affect all products manufactured within that platform. While it is important to confirm that changes to the platform do not impact the product, it is the responsibility of the manufacturer based on knowledge and understanding of the platform and the critical quality attributes to assess the change and to ensure through a control strategy that the implemented change did not result in a negative impact on product quality. Clarification is also provided for the assessment of the change following the point in the process for which the change was implemented. Modify as follows: "If a change is made to the ~~whole "~~platform ~~manufacturing"~~ process, then the effects ***downstream of the change*** for each concerned product should be evaluated separately. In principle, ***re-evaluation*** ~~revalidation~~ of the process for each separate product should be performed ***downstream of the change***, unless it can be justified that results can be extrapolated across the platform. ***Use of family, modular and bracketed approaches to validation is encouraged.***"	

4.3.2 Page 7 Lines 44-48 (EFPIA/EBE)	Type of comment: major Simultaneous submission of variations for all products in a platform would be difficult in situations with shared equipment and resources. Process validation or evaluation for all products cannot be executed within the same timeframes for simultaneous submissions. Additionally not all changes are reportable and so an additional sentence is added to clarify if a change is reportable. It is likely that each product will have a different assigned Rapporteur and therefore simultaneous submission of the same data to several reviews may result in significant duplication of effort and the potential for different questions to be asked on the same data, unless different Rapporteurs actively collaborated to consolidate a single set of questions, which could be difficult in the timeframe allowed for review. It should also be acknowledged that some older products may not be authorised via the Centralised Process and so simultaneous submission via two regulatory processes is also likely to result in duplicative/overlapping review cycles. Alternatively, and in view of the Commission's proposals to revise the variations regulations, EFPIA/EBE recommends that this paragraph is deleted, or that allowance is made within the proposed changes to the regulations for such submissions to be made by a single Variation applicable to multiple licenses. Once data have been generated in support of a manufacturing change for one molecule, risk to the other molecules in the platform is significantly reduced. We recognise that the current variations regulations do not permit use of Type 1A or 1B variation for manufacturing changes to biological products, but suggest that revision to address changes related to platform manufacturing might be considered in the ongoing review of those regulations. The proposal is to have the parent change assessed through the Type II Variation. The Type II Variation package would include the protocol that will be followed to assess the change for each individual product in the platform. Manufacturers should then be provided the opportunity to submit a 'minor' variation (Type IA/B in the current regulations) for submission of the platform manufacturing change to each additional product.	

	Proposed rewording: "***If the change requires regulatory notification,*** simultaneous submission of related Variations to the Marketing Authorisation for several products is ***optional*** to highlight changes to the "platform manufacturing" process. ***An alternative optional approach would be for applicants to request the appointment of a lead Rapporteur to assess the platform technology change. This assessment would thereafter be "mutually recognised" and leveraged by other agency reviewers for follow-up submissions for other affected products.*** Each variation should contain a comprehensive data package, including ~~relevant validation~~ data obtained with other monoclonal antibodies if the Marketing Authorisation Holder wants to extrapolate these data to the monoclonal antibody for which the Variation is submitted."	

4.3.2 Page 7 Lines 45-48 (PDA)	It is likely that each product will have a different assigned Rapporteur and therefore simultaneous submission of the same data to several reviews may result in significant duplication of effort and the potential for different questions to be asked on the same data, unless different Rapporteurs actively collaborated to consolidate a single set of questions, which could be difficult in the timeframe allowed for review. It should also be acknowledged that some older products may not be authorised via the Centralised Process and so simultaneous submission via two regulatory processes is also likely to result in duplicative/overlapping review cycles. An alternative approach may be for Regulators, in consultation with the applicant, to appoint a "lead" Rapporteur reviewer, to assess the platform technology change for a representative product, and thereafter "follow-up" submissions would be able to leverage the review of the platform technology and instead focus on any product-specific attributes of the change. Guideline should emphasise that such submission strategies are optional as defined by the marketing authorisation holder. Change to read: *"Simultaneous submission of related Variations to the Marketing Authorisation for several products is optional but recommended to highlight changes to the "platform manufacturing" process. An alternative optional approach would be for applicants to request the appointment of a lead Rapporteur to assess the platform technology change. This assessment would thereafter be "mutually recognised" & leveraged by other agency reviewers for follow-up submissions for other affected products. Each variation should contain a comprehensive data package, including relevant validation data obtained with other monoclonal antibodies if the Marketing Authorisation Holder wants to extrapolate these data to the monoclonal antibody for which the variation is submitted.*	
4.3.2 Page 7 Lines 47 (ROCHE)	Does this mean if we submit process changes we should bundle them together? Reading further in the paragraph it appears they want a clear justification and link to other validation data that will be used to support the new application. Clarification here would be helpful. From our point of view, the procedure of a simultaneous submission is not practicable in most of the cases.	

4.3.2 Page 8 Lines 1-4 (EFPIA/EBE)	Type of comment: major Reference to "partial revalidation" can be misinterpreted as written in the guidance. EFPIA/EBE suggests further clarity with regard to use of partial validation that would be acceptable. EFPIA/EBE recommends that "Platform assays" should be verified and/or validated for each new product against the relevant performance specifications defined for the method. Also it should be possible for verification to apply for certain sub-processes or single products on a worst case basis. Proposed rewording: "Platform assays" are equally acceptable; however, for each product at least a partial revalidation ***and/or one time verification*** to demonstrate suitability for the intended purpose should be performed (for example a platform assay for HCP might be feasible, especially if the parent cell line is identical for several products, but it should have the ***appropriate*** ~~same~~ (*) sensitivity and specificity for HCP's from different producer cell lines." (*): Change also proposed by PDA and ROCHE.	
4.3.2 Page 8 Line 4 (EFPIA/EBE) (HAS), (MSD) (MEDIMMUNE)	Missing a parenthesis: Add: ")" at the end of the sentence.	

4.3.3 Page 8 Line 6-12 (EFPIA/EBE)	Type of comment: minor Several suggested wording changes are proposed for clarification: • Cells from genetically modified animals other than mice may be used to produce mAbs • Replace "validation" in with "viral safety evaluation" to be consistent with Q5A • Source cells in this context are in reference to the host cell • Include reference to both enveloped and non-enveloped viruses • The use of platform technologies should be acknowledged for viral safety studies Proposed rewording: "Monoclonal antibodies derived from genetically ***modified animals*** ~~engineered mice~~ should also comply with ICH Q5A. The importance of ***a safety evaluation*** ~~validation studies~~ is emphasised. Source cells ***(eg, host cells)*** should undergo suitable screening for ***adventitious agents*** ~~exogenous agents and endogenous agents~~. The choice of viruses for the tests is dependent on the species and tissue of origin***, for both enveloped and non-enveloped viruses***. ***The use of platform technologies to support a reduced viral safety evaluation can be accepted, where justified by the applicant.***"	
4.3.3 Page 8 Line 10 (PDA) (ROCHE)	It would be helpful to just have one section dealing with viral validation. See paragraph 4 under 4.3.2. The use of platform technologies should be acknowledged for viral safety studies. Rephrase paragraph as follows: *"Viral safety aspects of monoclonal antibodies covered by this guideline should comply with ICH Q5A. Source cells should undergo suitable screening and virus safety evaluation shall be performed in accordance with current guidelines for biotechnological products. The use of platform technologies to support a reduced viral safety evaluation can be accepted, where justified by the applicant".*	

4.3.3 Page 8 Line 13 (EFPIA/EBE)	Type of comment: minor Suggested wording for clarification regarding when this guideline reference is applicable. "Where material of bovine or other TSE-relevant animal species have been used in development or manufacture ***of material for use in clinical studies***, the Note for guidance……..should be consulted."	

4.4. CHARACTERISATION OF MONOCLONAL ANTIBODIES

Page no. + Line no.	Comment and Rationale	Outcome
4.4 Page 8 Lines 17-30 (MEDIMMUNE)	This section needs an introductory statement about what is required for different stages of development, for example, indicating that heterogeneity will be better understood as development proceeds. New text proposed for insertion between line 21 and 22: "It is understood that more information about the characteristics of the protein will become available as data are generated during product development."	*Most of these comments were taken into account in the adopted document; however, the section on characterisation was completely reorganised to follow ICH Q6B headings, and unnecessary parts of the draft document were deleted.* *See adopted guideline for details.*
4.4 Page 8 Lines 22-24 (MEDIMMUNE)	Reference should be made to the fact that the characterization should relate to the mechanism of action, both in terms of physicochemical and biological characterization. New text: "The monoclonal antibody should be characterised thoroughly, including both its physicochemical and biological properties, <u>taking into account the mechanism of action. For example, a monoclonal antibody with effector functions should include more detailed characterization of the Fc region than is required for a Fab fragment or a monoclonal antibody demonstrated to lack effector function</u>. In addition, the specificity and cross-reactivity of the antibody should be assessed."	

4.4 Page 8 Lines 25 to 28 (EFPIA/EBE) (PDA) (ROCHE)	It should be taken into account that peptide mapping could be used in conjunction with mass spectroscopy or amino acid sequencing. N- and/or C-terminal sequencing is suitable for verification of the terminal amino acid sequences. In accordance with ICH Q6B, the sentence of line 27-28 should be changed to: "The sequence should be deduced by DNA sequencing and confirmed experimentally by peptide mapping ***or other appropriate analytical technologies."***	
4.4 Page 8 Line 27 (HAS)	Editorial: "The primary sequence.." is a bit inaccurate. Replace by "The primary structure….".	
4.4 Page 8, Line 27 (WYETH)	Additional analytical methods for experimental amino acid sequence confirmation should be allowed. Text revision is proposed: "*The primary sequence should be deduced by DNA sequencing and confirmed experimentally by peptide mapping and amino acid sequencing, or by other analytical methodology allowing for primary sequence verification (e.g. mass spectrometry)."*	
4.4 Page 8 Line 28 (MEDIMMUNE)	Peptide mapping, etc are examples of methods that could be used. New text: "...and confirmed experimentally showing data by, for example, peptide mapping, mass spectrometry, or other suitable methods."	
4.4 Page 8 Line 28 (MSD)	The term "peptide mapping" needs further definition. Is it only peptide mapping using HPLC or peptide mapping using LC-MS? Amino acid sequencing is redundant if LC-MS is run as it does not give additional information. It can be a complementary tool if we only run peptide mapping using HPLC. Revise line 28 to read "… peptide mapping by LC/MS or peptide mapping by HPLC and amino acid sequencing."	

4.4 Page 8 Lines 29-30 (EFPIA/EBE)	Type of comment: minor Clarify that "the following parameters" are not intended for routine release but are aspects of characterization only: "…the following ~~(additional)~~ parameters may be specifically relevant for monoclonal antibodies ***and should be performed for characterization only***."	
4.4.1.1 Page 8, Line 33 (WYETH)	Clarify the statement applies to "natural" or "non-engineered" IgG4 subclass antibodies. Wyeth engineers the hinge sequence of the IgG4 class antibodies to eliminate "half antibody molecules". Text revision is proposed: "*Characterisation should start with the determination of class, subclass, light chain composition and determination of the amount of half antibody molecules in case of a monoclonal antibody belonging to the <u>non-engineered</u> IgG4 subclass*."	
4.4.1.1 Page 8 Line 33 (EFPIA/EBE)	Type of comment: minor Modification as follows: "..light chain composition ***(kappa and/or lambda chain)***"	
4.4.1.1 Page 8 Line 35 (EFPIA/EBE)	Improve clarity: transfer lines 27-28 to the end of line 35.	
4.4.1.1 Page 8 Line 33-35 (MEDIMMUNE)	See comment from MEDIMMUNE on page 8, lines 22-24. Additional text to come after Line 35: "For antibodies where effector function may play a role in the mechanism of action, more emphasis should be placed on understanding the glycosylation patterns, in addition to the impact of product-related variants on biological activity."	
4.4.1.2 Page 8 Lines 38 Page 9 Lines 1-3 (EFPIA/EBE)	Type of comment: minor Consider to define "species" in "definitions" or to choose different term consistent with that proposed in ICH Q6B.	

4.4.1.2 Page 8 Line 39 (EFPIA/EBE)	Type of comment: minor Recommended clarification of "heterogeneity", which can mean aspects other than just charge, such as a varying population of antibody with heterogeneous glycoforms within a sample. Rewording: "Monoclonal antibodies therefore display considerable heterogeneity that can be characterised by several orthogonal methods. For example, charge heterogeneity may be characterised by isoelectric focusing (IEF), ion exchange chromatography (IEC), or capillary electrophoresis (CE)"	
4.4.1.2 Page 9 Lines 1- 4 (EFPIA/EBE)	Type of comment: major Full identification of all species is often not feasible and characterization to the extent possible should be discussed and justified. As described in ICH Q6B, understanding the biological activity of product-related variants provides important information relevant to the full understanding of the biological properties of the molecule. Proposed rewording: "The Applicant should characterise (biochemically and biologically) the possible discrete modifications and the major peaks seen in chromatograms; however, a full ~~identification~~ characterization of all the different minor species ~~will often~~ may not be feasible."	
4.4.1.2 Page 9 Lines 2-4 (MEDIMMUNE)	It is unclear how characterising all possible discrete modifications matches with characterising the major peaks. What if the modification is only a minor constituent? The definition of a major peak is also unclear, and without further detail would necessitate a judgement by the company. It is proposed to amend the text as follows: "The Applicant should, as far as is possible and relevant, characterise the discrete modifications and the major peaks seen in chromatograms by the time of licensure; however a full identification of all the different minor species will often not be feasible."	
4.4.1.2 Page 9 Line 4 (MSD)	Even the full identification is not possible; the consistency needs to be demonstrated. Add to the end of the sentence "… , in which case, product consistency in overall profiles of the different minor species should be demonstrated."	

4.4.1.2 Page 9 Lines 5-7 (EFPIA/EBE)	Type of comment: minor The use of "*specific"* implies that this could not happen for other proteins or fragments containing C-terminal lysine. Additionally, C-terminal Lys is not necessarily considered a product-related impurity. Proposed rewording: "A form of heterogeneity ~~very specific~~ ***common*** for monoclonal antibodies is C-terminal charge heterogeneity. Lysine residues from the C-termini are often partially or completely removed by a carboxypeptidase B-like activity. ***Although Lys-bearing forms are generally not considered product-related impurities*** (*)***, the potential presence of this modification should be assessed*** ~~the extent of Lys-removal should be addressed.~~ " (*): change also proposed by PDA and ROCHE.	
4.4.1.2 Page 9 Line 7 (MEDIMMUNE)	Not clear what is meant by "addressed".	
4.4.1.2 Page 9 Line 7 (WYETH)	We agree that the C-terminal heterogeneity needs to be addressed. It would be useful to know if there is a specific regulatory concern for this requirement (C-terminal Lys removal) for monoclonal antibodies. Wyeth monitors C-terminal heterogeneity for process and product consistency (as proposed in the text revision). Revised text: "*The extent of Lys removal should be addressed <u>for process and product consistency assessment</u>*."	

4.4.1.3 Page 9 Lines 9-13 (EFPIA/EBE) (PDA) (ROCHE)	Type of comment: major "*Typically, monoclonal antibodies have only one N-glycosylation site, on each heavy chain located in the Fc region, and the light chain is not glycosylated*": This statement is not correct because a significant number (~20%) of human immunoglobulins are glycosylated within the variable domains, see for example L. Huang et al. (2006) Analyt. Biochem. 349, 197-207. Additional references discussing glycosylation of immunoglobulin light chains: • B.J. Scallon et al. (2007) Mol. Immunol. 44, 1524 – 1534 • T. Martinez et al. (2007) Journal of Chromatography 1156, 183 -187 • Y. Fujimura et al (2006), Biosci. Biotechnol. Biochem. 64 (11), 2298 – 2305 Modify this sentence as follows: "Typically, monoclonal antibodies have ~~only~~ one N-glycosylation site on each heavy chain located in the Fc region, and the light chain is ***usually*** not glycosylated. ***There may also be an additional glycosylation site in the variable domain of the heavy chains.***"	
4.4.1.3 Page 9 Lines 9-13 (PDA) (ROCHE)	The document does not appear to reflect current literature on glycosylation. Fc glycosylation heterogeneity can be due to oligomannose forms vs. complex-type, extent of galactosylation, extent of fucosylation, sialylation (trace), alpha1-3Gal- and site occupancy. We recommend deleting the "degree of sialylation" phrase and rewording line 13: "*All glycan structures present should be fully characterized, paying attention to <u>those that affect biological activity of the monoclonal antibody."</u>*	
4.4.13 Page 9 Line 9 (LUDGER)	Some monoclonals can bear Fab glycosylation as well as Fc glycosylation. Proposed rewording: "*Most monoclonal antibodies have only one N-glycosylation site, on each heavy chain located in the Fc region, and the light chain is not glycosylated. <u>Some antibodies can also bear Fab glycosylation with variable levels of occupancy of the glycosylation site.</u>*"	

4.4.1.3 Page 9 Lines 10-12 (EFPIA/EBE)	Type of comment: major As a general comment to note in this section, not all antibodies as described within the context of this guideline would require characterization of the glycan structures, for example, some antibody proteins such as fusion proteins are expressed from bacterial cell lines or other non-glycosylating systems; therefore, it is suggested that reference be made to characterization of those antibodies that are glycosylated. Additionally, some IgG's have been shown to have unusual glycosylation patterns and this scope should also not be limited when considering alternate expression systems. Modify this sentence as follows: "The number of glycan structures typically found in most IgG's is limited and these structures are ~~primarily~~ ***usually*** biantennary with ***varying levels of terminal galactose.***"	
4.4.13 Page 9 Lines 11-12 (LUDGER)	No mention of fucosylation which is critical for some IgG1 Fc effector functions - e.g. ADCC. Proposed rewording: "*The number of glycan structures found in most IgGs is limited and are primarily biantennary with so-called G0, G1, and G2 glycan structures which have a variable presence of terminal galactose as well as oligosaccharides with and without core fucose.*"	
Section 4.4.1.3 Line 12-13 (EFPIA/EBE)	Type of comment: major The document does not appear to reflect current literature on glycosylation. Fc glycosylation heterogeneity can be due to oligomannose forms vs. complex-type, extent of galactosylation, extent of fucosylation, sialylation (trace), alpha1-3Gal- and site occupancy. We recommend deleting the "degree of sialylation" phrase and modifying lines 12-13: Modify this sentence as follows: "All ***major*** glycan structures ~~present~~ should be as fully characterized as ***technologically feasible, and the potential safety and efficacy impact of the glycan structures should be assessed.***"	

4.4.13 Page 9 Lines 12-13 (LUDGER) (LFB)	The levels of the different types of sialic acid residue (e.g. human-type NeuAc vs non-human NeuGc) are important for glycan characterisation of biopharmaceuticals. Proposed rewording: *"All glycan structures present should be fully characterised, paying attention to the degree <u>and type</u> of sialylation."*	
4.4.13 Page 9 Line 12-13 (MEDIMMUNE)	It is not practically feasible to characterise all glycan structures fully. The following structures have all been identified on mAbs using modern highly sensitive mass spectrometric methods: Neutral glycans: G0, G0F-GN, G0F, G1F, G2F, G3F Acidic glycans: G1FA1-GN, G2FA1, G3FA1 However, only G0F, G1F and G2F are typically seen at abundances greater than 2% - that is all the others are 2% or less of the total oligosaccharide present. Thus, fully characterising such low levels of these species is not accurate or reproducible. Furthermore, characterisation of glycan structures should only include details regarding the linkage where this is known to have an impact on the biological activity. Revised text: "The three main glycan structures present (G0, G1 and G2) should be characterized as fully as possible and relevant, paying attention to the degree of sialylation, where appropriate. Full characterization of both major and any additional minor oligosaccharide species will not always be practical."	
4.4.1.3 Page 9 Line 13 (MSD)	Glycans are fucosylated most of the time. The extent of defucosylation needs to be monitored. Add to the end of the sentence "… and fucosylation"	
4.4.1.3 Page 9 Line 13 (WYETH)	We agree that the glycan structures need to be fully characterised. It would be useful to know if there is a specific regulatory concern regarding the degree of sialylation of monoclonal antibodies.	

4.4.2, 4.4.3 Page 9 (EFPIA/EBE)	Type of comment: major Section 4.4.2 Biological /immunological characterisation and Section 4.4.3 Specificity and Cross reactivity studies are appropriately referred to in the ICH S6 document and are not related to the production and quality control aspects of monoclonal antibodies. EFPIA/EBE members strongly recommend the deletion of these sections from this guidance document; in the event that these sections are deemed essential, significant changes are required for clarity as noted below.	

4.4.2 Page 9 Lines 15-24 (EFPIA/EBE)	Biological/immunological characterisation should be attempted, but it may not be possible to obtain all the listed properties in all cases; rewording for clarification is suggested. - Additional study parameters should also include the bioactivity activity assessment *in vitro* and/or *in vivo* assays, depending on its activity beyond the affinity analysis. Kd is one form of affinity analysis and thus should not be listed separately. - The characterisation of the epitope may not be feasible for all monoclonal antibodies. Defining the epitope rarely tells you about specificity unless it happens to be a linear epitope and even then it's not that predictive. Because many antibodies recognise non-linear epitopes, it would be almost impossible to meet this requirement in many situations. - For humanized antibodies, paratope definition can be limited to CDR-regions. Analysis may not add value beyond confirming that the CDR regions are important for binding and should be evaluated as needed. - The paratope is generally known because the CDR is the region that is defined during development as the antigen binding moiety on the antibody. Use of "Paratope" needs to be defined to eliminate ambiguity. The identification of the paratope is only possible by in depth structural biology studies (example: crystallographic methods) which are very difficult to perform, and in our opinion will not provide relevant additional information for biological/immunological characterisation. - What are acceptable tools for immunoreactivity predictions? - Immunoreactivity has been removed from this section. It would be helpful to provide examples to more clearly define agency expectations. - It should be taken into account that for many monoclonal antibodies, the mode of action is not cytotoxicity and the mode of action via the Fab may be binding of soluble antigen - It should also be noted that effector function assays need to be addressed for specific isotypes using effector cells only if these activities are important for the mechanism of action of the therapeutic. Measurement of complement binding may not be feasible if the antibody is directed against a soluble target.	

	Proposed rewording of section 4.4.2: "The immunological properties of the antibody should be described ~~in detail~~ and ***may include any of the following properties as relevant:*** • ***biological activity assessment in in vitro and/or in-vivo assays.*** • affinity ~~and Kd~~; • antigenic specificity including the characterisation of the epitope that the antibody recognizes***, where feasible*** • the ~~paratope~~ CDR-regions (the part of the monoclonal antibody that recognises and binds to the epitope) should be identified, if relevant • the *in-vitro* immunoreactivity of the purified monoclonal antibody." When relevant to mechanism of action these additional characteristics should be described: • antibody-dependent cytotoxicity (ADCC), cytotoxic properties (apoptosis), ***as appropriate*** • ability for complement binding and activation and other effector • functions, ***as appropriate*** • ***Fc gamma receptor binding activity*** • ***FcRn binding activity*** • ***Agonist or antagonist activities, if any*** • ***The CDR-regions (the part of the monoclonal antibody that recognises and binds to the epitope) should be identified, if relevant***." Type of comment: minor Lines 23-24: We recommend omitting "*the specific activity of the purified monoclonal antibody should be determined (units of activity/mass of product)"*. This is more appropriately described in the potency section 4.5.2.	

4.4.2 Page 9 Line 15-24 (MEDIMMUNE)	It should be pointed out that the extent of epitope and paratope mapping should be dependent on the phase of development of the product. Suggested wording for revised section 4.4.2: "The biological / immunological properties of the antibody should be described in detail and should include: - Binding properties: o Antigenic specificity including the characterisation of the epitope that the antibody recognises; o Affinity and Kd; o Identification of the paratope (the part of the monoclonal antibody that recognises and binds to the epitope). The extent of epitope and paratope mapping will be dependent on the stage of development. Information should be available by the time of licensure; and o Immunoreactivity of antibody as determined by non-clinical data. -Effector functions as applicable: o Ability to activate complement binding and activation; and o Other effector functions."	

4.4.2 Page 9 Lines 15-24 (PDA) (ROCHE)	The immunological properties of the antibody should be described in a context dependent manner. *- the paratope (the part of the monoclonal antibody that recognises and binds the epitope) should be identified* The paratope identification is not a matter for this guideline which addresses production and quality control topics. The section should be addressed in non-clinical guidelines. In addition it is not clear for what purpose this point is addressed – this point should be deleted; The characterization of regions important for binding are covered by other methods (e.g. BiaCore) Reword as follows: *"For all monoclonal antibodies the following characteristics should be described:* • *affinity and/or Kd;* • *antigenic specificity including the characterisation of the epitope that the antibody recognizes where feasible* *When relevant to mechanism of action these additional characteristics should be described:* • *antibody-dependent cytotoxicity (ADCC), cytotoxic properties (apoptosis)* • *ability for complement binding and activation and other effector functions (CDC);* • *Fc gamma receptor binding activity* • *FcRn binding activity* • *Agonist or antagonist activities, if any"*	

4.4.2 Page 9 Line 15 (WYETH)	Recombinant monoclonal antibodies and related substances can be adequately characterised by modern state-of-the art physicochemical methods. Therefore, the characterisation criteria that apply to protein pharmaceuticals should be sufficient for determination of recombinant monoclonal antibody structure/heterogeneity and require only limited "immunological" characterisation. The immunological properties listed are suggested as relevant examples rather than a requirement. Text revision is proposed. Inclusion of "the immunoreactivity of the antibody" (line 22) is unclear in terms of recombinant protein characterization. If this were a safety concern (immunogenicity), it would be better addressed through clinical studies not by protein characterisation. Revised text: "*The immunological properties of the antibody should be described and may include:* *- antigenic specificity including the characterisation of the epitope that the antibody recognizes;* *- antigen binding characteristics (affinity and Kd);* *- the paratope (the part of the monoclonal antibody that recognizes and binds to the epitope).*"	
4.4.2 Page 9 Lines 18-19 (PDA) (ROCHE)	The ability for complement binding and activation as well as ADCC should not only be described, but quantified using appropriate assays. This is not a "go/no go" situation. Combine lines 18 & 19 and add "*these properties should be quantified using appropriate assays"*	
4.4.2 Page 9 Line 18 (HAS)	This point might be more specific. Replace by: " - ability for complement binding and activation, and interaction with cell surface Fc-receptors"	
4.4.2 Page 9 Lines 20-21 (LFB)	There is no rationale and no interest to identify the paratope. It is a complex and heavy burden to identify the paratope; crystallography studies would be necessary. We demand to suppress this sentence: *"the paratope (…) should be identified."*	

4.4.2 Page 9 Lines 20-21 (PDA)	*"… the paratope (the part of the monoclonal antibody that recognises and binds the epitope) should be identified…"* It is not clear for what purpose this point is addressed. The characterization of regions important for binding is covered by other methods (e.g. BiaCore). This text should be deleted.	
4.4.2 Page 9 Line 22 (PDA)	Regarding immuno-reactivity of the antibody, it would be helpful to provide examples to more clearly define agency expectations. Is detection of HAHAs (Human anti-human antibodies) sufficient, or are other species envisioned?	
4.4.2 Page 9 Line 22 (PDA) (ROCHE)	We recommend omitting "*the immunoreactivity of the antibody"* from this section. This statement is redundant as this is described in section 4.4.3.	
4.4.2 Page 9 Line 23 (MEDIMMUNE) (PDA) (ROCHE)	We recommend omitting "*the specific activity of the purified monoclonal antibody should be determined (units of activity/mass of product)".* This is more appropriately described in the potency section 4.5.2.	
4.4.2 Page 9 Line 23 (WYETH)	Some attributes described under "Immunological" properties (lines 18, 19) better fit into "Biological" properties. Propose to move the text to line 23/24. Revised text: "*The specific activity of the purified monoclonal antibody should be determined (units of activity/mass of product). <u>The ability for complement binding and activation, and other effector functions should be evaluated and included, if appropriate in the bioactivity testing (e.g. antibody-dependent cytotoxicity and other cytotoxic effect).</u>"*	

4.4.3 Page 9 Lines 25-34 (PDA) (ROCHE)	Complete paragraph should be deleted due to the following reason: The epitope determination as well as investigations on cross reactivity is not a matter for this guideline which addresses production and quality control topics. The section should be addressed in non-clinical guidelines. In the description given in this section it is also not clear at which time point in development the investigations should be performed? Part of the preclinical investigations?	*The recommendation (in the adopted document) regarding crossreactivity is in line with ICHQ6B. It is acknowledged that the list of suggested tissues to be tested for crossreactivity should be addressed in non-clinical and/or clinical guidance. However, since this list is currently not available in any other European guidance, it was maintained as an annex to this document, which may be updated or removed in the future, as necessary.*
4.4.3 Page 9 Line 26 (HAS)	The first sentence repeats the 1st bullet point of 4.4.2. A rewording is proposed: "Beyond determining the epitope primarily recognised by the monoclonal antibody, the analysis of its binding activity should further include the determination of unintentional…..".	
4.4.3 Page 9, Lines 26-27 (LFB)	This point should be reconsidered. In many cases the epitope is not a linear sequence of amino acids but a conformational structure. It is difficult to determine such a sequence. That s' why we propose to amend this point. Proposed rewording: *"The epitope should be identified (nature of the molecule: protein, oligosaccharide, glycoprotein, glycolipid…) and if possible characterized (amino acid sequence)."*	
4.4.3 Page 9 Lines 26-27 (MSD)	The sentence needs to be reworded, since "determining the epitope recognized by an antibody" is sometimes not a simple task (assuming that it is always doable). This is highly dependent of what one defines as "determined" at either AA level or AA-equivalent structural level. Suggest that the guidance should emphasize and accept good data on demonstrating specificity and cross-reactivity. Add to the end of the sentence on line 27 "… when feasible"	
4.4.3 Page 9 Line 26 (WYETH)	Section applicable to non-clinical evaluation rather than production and quality control. The non-clinical evaluation is contained in a different section of the CTD, and therefore inconsistent with the focus of the guideline. The epitope determination (specificity) has been already covered in section 4.4.2. Propose to remove the section 4.4.3.	

4.4.3 Page 9 Lines 26-29 (EFPIA/EBE)	Type of comment: major The analysis of unintended cross reactivity with, or cytotoxicity to, human tissues is not a quality issue. It is a safety issue that is addressed in ICHS6. This type of cross reactivity assessment is distinct from affinity analyses comparing antibody affinity for the intended target and closely related members of the same gene family (which can be referred to as a cross reactivity assessment), or other types of in vitro assessments that are intended to define the immunoreactivity of an antibody. EFPIA/EBE members strongly agree that these related sections should be deleted from this document since these details are not in scope of this document on product quality and control. If these sections remain, the following re-write is suggested for lines 26-29 to technically correct the statement: ***"The target molecule bearing the relevant epitope should be biochemically defined and the epitope (amino acid sequence or equivalent structural moiety) recognized by the monoclonal antibody should itself be defined, if feasible. Binding assays of the antibody to purified antigens and defined regions of antigens should be performed, as feasible, to determine affinity, avidity and immunoreactivity (including in vitro cross-reactivity to other structurally homologous proteins)."***	

4.4.4 Page 9 Line 35 (MEDIMMUNE)	A more appropriate section heading may be “Particulates”	*The section on particulates has been significantly reworded to take into account the comments received, and the discussions during the Industry/BWP meeting. All methods and acceptance criteria initially mentioned have been removed.* *See adopted guideline for details.*

4.4.4 Page 9 Lines 35-46 (PDA)	"(often >100 mg/vial)" implies vial presentation and therefore we propose removal of "(often >100 mg/unit)". Examples of quantity of high Mab protein and its correlation to particulates as a "natural tendency…to aggregate" is troubling. Consideration to the different techniques in characterizing and differentiating soluble proteinaceous particles, from a discussion regarding foreign particulate matter in parenteral products is recommended. Visible and sub-visible insoluble foreign extraneous particulate matter only should be assessed using current Ph Eur methods. Therefore, this should be specified on page 11 in section 4.5.6 to replace lines 29-33 and not in the characterization section. Additionally, the statement "*The presence of such visible particulates is unwanted*." can be interpreted as a specification of "Zero". Since there is a reference to specific regulations for foreign particulates, we suggest this statement be deleted. Delete also from p. 11. Proposed rewording of this paragraph as follows: "*High concentrations of monoclonal antibody are often necessary to obtain a therapeutic effect, and therefore the concentration of monoclonal antibody protein in the final formulation are higher than for other biotechnological products. Because of their high amounts and tendency to form intrinsic soluble proteinaceous particles (including aggregates) in the final formulation, appropriate studies should be performed to find an optimal formulation that is stable with respect to formation of intrinsic proteinaceous particles including aggregates at release and during storage. Soluble proteinaceous particles including aggregates can be characterized for example using SEC with laser light scattering or AUC. Such methodologies should be employed during product development and characterization to assess the effects of formulation and environmental factors on protein aggregation and the relationship between formation of soluble proteinaceous aggregates and potential insoluble particulates".*	

4.4.4 Page 9 Lines 36-37 (EFPIA/EBE)	Type of comment: major This statement should not be expressed in absolute terms since not all antibody products are formulated at high concentration. Modify this sentence as follows: "~~High amounts of~~ ***The concentration of*** monoclonal antibody ***protein*** ~~are often necessary to obtain a therapeutic effect, and therefore the amounts concentrations of monoclonal antibody protein~~ in the drug product is ***typically*** higher than for other biotechnological products. ~~(often > 100 mg/vial)~~."	

4.4.4 Page 9 Lines 38-41 (EFPIA/EBE)	Type of comment: critical Whilst it is true to some extent that monoclonal antibodies are prone to particulate formation, this is not necessarily a significant issue. Furthermore, whilst it is agreed that optimal formulation development should evaluate particle formation and attempt to deliver a formulation that avoids their presence, often times this may not be possible, despite significant efforts on the part of the manufacturer. It is important that particulate matter is identified, characterised and quantified, and that the kinetics of formation is understood. Proteinaceous particles are frequently transient, reversible, and may or may not increase over time. Additionally there is not a correlation between the formation of visible particulates and aggregates. Proteinaceous visible and sub-visible particles may be inherent to some high concentration protein and antibody formulations Also to note, the presence of sub-visible particulates does not necessarily mean they will aggregate to form visual particulates. The statement "natural tendency…to aggregate" is somewhat troubling and is very dependent on the product and formulation. The statement, "The presence of such visible particulates is unwanted." is contradictory to the EP monograph 0520 and established regulatory guidance, which states that injections are 'practically free from particles". Since there is a reference to specific regulations for particulates, we suggest this statement be deleted from here and also from line 33 on page 11. It is suggested to remove reference to aggregates since there has not been a correlation. Modify this sentence as follows: "~~Because of the higher amounts and a natural tendency for immunoglobulins to aggregate,~~ The formation of sub-visible and visible particulates ~~(including aggregates~~) in the drug product ~~is a significant~~ ~~issue~~ ***is of importance and should be investigated*** ***and closely monitored on lot release and during stability studies***. ~~The presence of visible particulates is unwanted.~~ "	
4.4.4 Page 9 Line 38 (HAS)	Editorial: more accurate wording is proposed: "…the high concentrations and a natural tendency…"	

4.4.4 Page 9 Lines 38-40 (ROCHE)	"high amounts" of what - antibody product or aggregate? The statement "natural tendency…to aggregate" is troubling. This seems to imply antibodies are just waiting to jump out of solution. Reword this sentence: *"…, and therefore the concentration of antibody in the final formulation may be higher than normally experienced for other biotechnology products, especially in the case of sub-cutaneous delivery forms. The formation of sub-visible and visible particulates is therefore of importance and should be closely monitored on lot release and during stability studies. The presence of visible particulates is unwanted…."*	
4.4.4 Page 9 Lines 41-44 (EFPIA/EBE)	Type of comment: critical The referenced pharmacopoeial monographs were developed to address the potential presence of extrinsic foreign matter in small molecule drugs, and it is patently inappropriate to apply these criteria to intrinsic proteinaceous particles. Proteinaceous visible and sub-visible particles may be inherent to some high concentration protein and antibody formulations despite efforts to limit their formation through formulation development. In such cases product quality, clinical experience, efficacy, safety and immunogenicity considerations should be taken into account to determine acceptability of such particle containing formulations. Generalised pharmacopoeial requirements are clearly inappropriate where such a case by case assessment is indicated. Additionally, product administered to patients through in line filters should be exempt from the requirements providing the filter delivers a solution complying with the requirements of 2.9.19. Modify section as follow: "Unless otherwise justified, the drug product should ~~always~~ comply with the requirements set forth in the Ph. Eur. Monograph on "Parenteral preparations" (~~07/2005~~:0520): 2.9.19. Particulate contamination: sub-visible particles (~~01/2005~~:20919). ~~and other pharmacopoeial requirements on visible particles.~~ Appropriate formulation studies should be performed to find ~~an optimal~~ ***a suitable*** formulation that is stable with respect to formation of particulates at release and during storage."	

4.4.4 Page 9, Line 41 (LFB)	*"The presence of such visible particulates is unwanted "*: Does this recommendation also include the sub-visible particulates?	
4.4.4 Page 9 Line 41 (ROCHE)	The statement "*The presence of such visible particulates is unwanted*." can be interpreted as a specification of "Zero". Since there is a reference to specific regulations for particulates, we suggest this statement be deleted. Delete also from p. 11, l 33.	
4.4.4 Page 9, Lines 42-43 (LFB)	Application of the Ph. Eur. sub-visible assay is ambiguous. It is clearly stated in the Ph. Eur. Monograph on "Parenteral preparations" (07/2005:0520) (cross-referenced in this guideline) that this test is applicable for preparations for human use, solutions for infusion or solutions for injection supplied in containers with a nominal content of more than 100 ml while the 2.919 Particulate contamination: sub-visible particles (01/2005:20919) both describes assays applicable to volumes of more and less than 100 ml. Could the application of the Ph. Eur. Sub-visible particulates assay with regard to the solution's volume be clarified in this guideline?	
4.4.5 Pages 9-10 (EFPIA/EBE)	Type of comment: minor The text regarding use of Units appear contradictory to the recommendations to use specific activity as stated under paragraph 4.4.2 and 4.5.2 and the text is somewhat unclear as to the recommendation. Suggest removal of this section to eliminate ambiguity.	*Comments taken into account in the adopted document.*
4.4.5 Page 9 Line 47 (MEDIMMUNE)	Filling based on mass should be moved to the introduction of section 4.5 "Specifications".	

4.5. SPECIFICATIONS		
Page no. + Line no.	**Comment and Rationale**	**Outcome**

4.5. Pages 10-11 (EFPIA/EBE)	It is recommended to add a section on "Purity" for alignment with ICH Q6B, with specific considerations for monoclonal antibodies.	*The section on specification was completely reorganised to follow ICH Q6B headings and principles.* *References to specific tests were removed from the document, or only cited as an example, to allow flexibility in the selection of methods, and take into account future technology evolutions.* *General references to Ph. Eur. monographs were maintained in the document, however the tests and limits described in these documents were not repeated in this guideline.*
4.5 Page 10 (MEDIMMUNE)	Revise this text to include reference to the need to make characterisation relevant to both physicochemical and biological properties. New text: "Appropriate release and shelf life specifications for both drug substance and drug product should be defined based on the physicochemical and biological properties identified during characterization of the antibody. The principles described in ICH Q6B should be used."	

4.5.1 Page 10 Lines 10-17 (EFPIA/EBE)	Type of comment: major This section needs to be reworded to clarify the text and provide technical accuracy and consistency. Clarification is required to define the intent for ID testing using a combination of tests. The text, "by a combination of tests with sufficient specificity (e.g. a specific ELISA which also determines potency)" is confusing. This is not a combination of tests, but rather a single test with two purposes. In reality an ELISA does not determine potency in all cases (e.g. where effector function is required for activity), as a "dead" antibody can still bind in an ELISA. This section needs to be discussed further and it should be clarified why it is not considered sufficient to utilise a single specific ELISA ID test based on binding to the CDR region of the antibody product. A single IEC method should also be acceptable as measure for identity. Further clarification is required to distinguish between testing differences required for drug substance vs. drug product. Recommend re-write: "Identity can be determined by one very specific test (eg. Peptide mapping, ***product-specific EIA, or a charge-based separation, such as IEF or cIEF, that clearly distinguishes the antibody from others likely to be manufactured in the same facility***) or a combination of tests with sufficient specificity to ***clearly identify the antibody (eg. Charge-based separation paired with a ligand binding assay*** or a specific ELISA which also determines potency***)***. ***It should also be noted that the identity testing requirements for bulk drug substance may be different from that for final drug product, and these requirements may dictate that different identity tests are appropriate for these two different sample types.*** However, the Applicant should justify that identity is demonstrated by the tests included in the specification, and that ~~mix-ups with other monoclonal antibodies can be ruled out. The latter aspect in particular is important for manufacturers that product several monoclonal antibodies at the same facility.~~ ***the chosen methods will uniquely identify each antibody manufactured within the same facility.***"	*As for any product/process, alternative approach to development and product testing, taking into account concepts described in ICH Q8/Q9/Q10, may be considered, if appropriately documented. As a consequence, these concepts were not specifically included in the document to avoid confusion with current ICH work.* *See adopted guideline for details.*

4.5.1 Page 10 Lines 11-12 (PDA) (ROCHE)	*"Identity can be determined by one specific test (e.g. peptide map) or by a combination of tests with sufficient specificity (e.g. a specific ELISA which also determines potency)* " Change of wording for clarifying the content: "*Used test for identity testing should be specific, e.g. peptide map or other appropriate method. If necessary a combination of methods should be used."*	
4.5.1 Page 10 Line 12 (MSD)	ID testing: in addition to peptide mapping, CE-IEF can be used for ID. Modify line 12 to read "… peptide mapping, CE-IEF)…".	
4.5.1 line 12 4.5.2 line 31 Page 10 (HAS)	Although frequently used, ELISA is only one of the binding tests. A more general wording is proposed: ELISA should be replaced by "binding test".	
4.5.1 Page 10 Lines 12-13 (MEDIMMUNE)	An ELISA suitable for identity is not necessarily suitable for potency determination. Revised text: "…sufficient specificity (e.g. a binding ELISA)."	
4.5.6 Page 11 Lines 19-20 (EFPIA/EBE)	Type of comment: major The relationship between this new guideline and the specifications in ICH Q6B and EP Monograph for monoclonal antibodies should be clarified and aligned. Additionally, the selection of tests to be included in the specification is product specific; therefore, not all of the "other" release tests listed in lines 21-33 will apply to all products. Modify lines 19-20 as follows: "Besides pharmacopoeial and other appropriate tests as outlined above and in ICH Q6B, ***tests for the following parameters may be considered as appropriate, depending on the specific nature of product***:"	

4.5.6 Page 11 Lines 19-28 (MEDIMMUNE)	Suggested change to text for clarity and consistency with the Ph.Eur. monograph on "*Monoclonal antibodies for human use*" (07/2005:2031). Molecular size distribution is related to heterogeneity and therefore should be in Section 4.5.4. Half-antibody determination should be included as a characterisation test and not a release test. It should be recognised that this will potentially only be semi-quantitative as NR-SDS PAGE (the only currently available method) is inherently variable. Information moved to Section 4.4. Suggested text to come after Line 28: "In addition to the tests outlined above, tests for: -appearance, -solubility, -pH, -osmolality, -extractable volume, -total protein, -sterility, -bacterial endotoxins, and -stabiliser and water, if appropriate, should be conducted as outlined in the Ph.Eur. monograph on "*Monoclonal antibodies for human use*" (07/2005: 2031). The structural integrity of modified (conjugated) monoclonal antibodies requires special consideration."	
4.5.6 Page 11 Lines 22 and 30 (EFPIA/EBE)	Abbreviation "SEC-HPLC" contains "chromatography" twice and thus is not the correct short form of "size exclusion high-performance liquid chromatography". Suggest that this acronym be changed to "SE-HPLC" (or alternatively "SEC").	

4.5.6 Page 11 Lines 23-24 (EFPIA/EBE)	Type of comment: minor The sentence "…*the structural integrity of modified (conjugated) monoclonal antibodies requires special consideration*" is vague. Since there is a later section on conjugates, it is not clear why this comment appears here, especially without additional information. Structural integrity can be studied by reduced/non-reduced SDS-PAGE with a serial-diluted sample (eg. IgG4 artefacts). Suggest adding SDS-PAGE as an example of a readily available test method for structural integrity and artefact detection.	
4.5.6 Page 11 Lines 25-26 (EFPIA/EBE)	Type of comment: minor It is stated that, for IgG4 isotype antibodies, a specification test for half antibody should normally be included. Provision should be added that if the IgG4 structure has been engineered to eliminate half-antibody formation and it has been demonstrated that half-antibodies do not form, a specification should not be necessary. Modify lines 25-26 and add sentence as follows: "For IgG4 isotype monoclonal antibodies, *the relative percentage of half-antibody detected during characterization should be considered. However, if the IgG4 structure has been modified to eliminate half-antibody formation and it has been demonstrated that half-antibodies do not form, a specification may not be necessary."*	
4.5.6 Page 11 Lines 25-26 (PDA) (ROCHE)	Sentence should be reworded as follows: *"For IgG4 the relative percentage of half-antibody detected during molecule size distribution characterization should be addressed."*	
4.5.6 Page 11 Line 25 (WYETH)	Clarify the statement applies to "natural" or "non-engineered" IgG4 subclass antibodies. Wyeth engineers the hinge sequence of the IgG4 class antibodies to eliminate "half antibody molecules". Text revision is proposed. Revised text: "*-For non-engineered IgG4 isotype monoclonal antibodies, a test for the amount of half antibody should normally be included*."	

4.5.6 Page 11 Lines 25-26 (PDA)	It is stated that, for IgG4 isotype MAbs, a specification test for half antibody should normally be included. Provision should be added that if the IgG4 structure has been engineered to eliminate half-antibody formation and it has been demonstrated that half antibodies do not form, a specification should not be necessary. Add the following after line 26: *"If the IgG4 structure has been modified to eliminate half-antibody formation and it has been demonstrated that half-antibodies do not form, a specification may not be necessary."*	

4.5.6 Page 11 Lines 29-33 (EFPIA/EBE)	Type of comment: major AUC is a useful characterization tool but is not a feasible method to validate and operate routinely; therefore, AUC should be considered a characterization method to support orthogonal methods that monitor size variants. AUC is very tedious and only covers a specific size range. You can also use HIAC, DLS, MALLS, FFF, UV absorbance etc. as orthogonal methods to SE-HPLC to cover the size range for particulates. They will not always be in the range of AUC every time. It is also important to differentiate particulates from soluble aggregates, in addition to the fact that the particulates in a sample may not actually be the monoclonal antibody itself. Lines 29-33 should be modified and moved to Section 4.4, "Characterisation of Monoclonal Antibodies". Additionally, a distinction should be made between particulate matter and soluble aggregates. Reference to both SE-HPLC and AUC in the context of analytical detection of particulate matter should be deleted because neither of these methods is intended to detect and characterize particulate matter. While it may be useful to discuss the limitations of SE-HPLC in detecting soluble aggregates and the utility of analytical ultracentrifugation (AUC) as an orthogonal method to detect soluble aggregates, it needs to be emphasized that the current state-of-the-art of AUC precludes its use as a release test and that its utility is to ensure comprehensive characterization of the aggregate. Modify this section (lines 29-32) and move to Section 4.4: "*In addition to the pharmacopoeia test for particulate matter, other analytical methods may be applied to determine levels of particulate matter. Orthogonal methods may be necessary to characterise soluble and insoluble aggregates and/or particulates. This would be determined on a case-by-case basis.*"	

4.5.6 Page 11 Lines 29-33 (MEDIMMUNE)	Visible particulates, by definition, are insoluble protein aggregates that cannot enter the pores of a HPLC column and thus cannot appear in the void volume. Secondly, AUC cannot be used to isolate or characterise particulates - they will spin down in the centrifuge. AUC is only useful for characterisation of soluble aggregate i.e. dimer or trimer which is still in solution and not precipitated to particulates. Revised text: "The analytical determination of particulate matter in the drug product raises difficult issues in practice. SEC-HPLC methods may not be suitable. Visible particulates can be trapped in the column pre-filter or removed prior to analysis (i.e. by sample pre-treatment with filtration or centrifugation); or they may disintegrate due to shearing when the sample is injected. Analytical ultracentrifugation may be useful to characterise soluble multimeric forms of the drug product i.e. dimer or trimer."	
4.5.6 Page 11 Lines (29-33) (PDA)	Visible and sub-visible **insoluble** foreign extraneous particulate matter only should be assessed using current Ph Eur methods. SEC-HPLC and AUC use should be described in section 4.4.4 for the characterization of soluble proteinaceous particles including aggregates. See our proposal for that section. Clarification is required as to the reference to the Ph Eur for parenterals since these criteria are based on extraneous foreign matter, not with reference to inherent or intrinsic proteinaceous soluble particles specific to the antibody formulation. Proposed statement is as follows: *"Visible and sub-visible **insoluble** foreign extraneous particulate matter in drug product should always comply with the requirements set forth in the Ph. Eur. Monograph on "Parenteral preparations" (07/2005:0520): 2.9.19. Particulate contamination: sub-visible particles (01/2005:20919) and other pharmacopoeial requirements on visible particles".*	

4.5.6 Page 11 Lines 31-32 (PDA) (ROCHE)	What about other methods such as field-flow fractionation and light scattering? Proposed rewording: *"Analytical ultracentrifugation, field-flow fractionation and light scattering may be more suitable to isolate and characterise particulates"*	
4.5.6 Page 11 Lines 32-33 (EFPIA/EBE)	Type of comment: minor Recommend deleting redundant information: delete the sentence that starts at the end of line 32 "As noted in……formulation development" to be consistent with the changes recommended in section 4.4.4, Formulation.	
4.5.1 Page 10 Line 13 (HAS)	The repertoire of possible identity tests may be extended. Insert to the end of the paragraph: "A validated anti idiotype reagent may also be a useful tool."	
4.5.4 Page 11 Lines 2-8 (EFPIA/EBE)	Type of comment: minor Release specifications should be based on critical quality attributes, i.e., those that are critical to safety and efficacy. Requirement of a specification to ensure consistency in heterogeneity may not be applicable, depending on the activity of the heterogeneities. Suggest modified of section as follows: *"Specification for heterogeneity should focus on heterogeneities related to decrease in activity. If product variants possess similar activity, specification should not delineate between variants. The specification should assure that the mixture of the species has a consistent pattern."*	
4.5.5 and 4.5.6 Page 11 Lines 9-33 (PDA)	We recommend that Page 11 lines 10 to 33 are moved to the section 4.4 characterisation.	

4.5.5 Page 11 Lines 10-17 (EFPIA/EBE)	Type of comment: major This general proposal for specifications on process-related impurities potentially disagrees with certain aspects of ICH Q6B Section 2.3 (Process Controls), particularly Section 2.3.2 (In-process acceptance criteria and action limits). "*In-process tests are performed at critical decision making steps and at other steps where data serve to confirm consistency of the process during the production of either the drug substance or the drug product. The results of in-process testing may eliminate the need for testing of the drug substance or drug product*." Section 2.3.3 goes on to say the following: "*The use of internal action limits by the manufacturer to assess the consistency of the process at less critical steps is also important. Data obtained during development and validation runs should provide the basis for provisional action limits to be set for the manufacturing process. These limits, which are the responsibility of the manufacturer, may be used to initiate investigation or further action. They should be further refined as additional manufacturing experience and data are obtained after product approval*." The guideline should acknowledge the validity of not performing routine testing for process-related impurities where supported by appropriate process development and validation data. Therefore, we believe that Section 4.5.5 should be reworded. Replace lines 10-17 with the following: *"Process-related impurities should be monitored during development and controlled with specifications, as appropriate.* *An appropriate validation approach may be used in lieu of a specification, if justified. For marketing authorisation, data should be provided to demonstrate consistent removal of relevant process-related impurities to acceptable levels."*	

4.5.5 Page 11 Lines 10-17 (PDA) (ROCHE)	See comment below from EFPIA/EBE: "This general proposal for specifications on process-related impurities potentially disagrees with certain aspects of ICH Q6B Section 2.3 (…) Section 2.3.3 goes on to say the following (…)." We would recommend to specifically mention host cell proteins. Does the term "all reagents" refer to antibiotics, vitamins, process buffers etc. It could be helpful to explain the term "all reagents" by different examples. In addition the use of the term "and similar cell lines" must be better defined. Therefore, we believe that Section 4.5.5 should be reworded as follows: "*For certain impurities, testing of either the drug substance or the drug product may not be necessary and may not need to be included in the specifications if efficient control or removal to acceptable levels is demonstrated by suitable studies. Process validation can be sufficient to replace a drug substance specification for residual host cell proteins or other process-related impurities. For a consistency check of the performance of a purification process, in-process testing with appropriate limits may be suitable."*	
4.5.5 Page 11 Lines 10-17 (PDA)	Provision should be made to demonstrate process removal through validation for any reagents and if used, Protein A, in addition to residual DNA. A specification would not be necessary in those cases. This approach is consistent with the Quality by Design philosophy. Add the following after the first and second sentences in section 4.5.5: *"An appropriate validation approach may be used in lieu of a specification."*	
4.5.5 Page 11 Lines 10-13 (MEDIMMUNE)	Edits in wording to add more detail around the appropriateness of including a Protein A specification. "For monoclonal antibodies, a specification for residual protein A should be considered if protein A chromatography is part of the purification process, based on the principle that it is a relevant process-related impurity. Additionally, it will serve as an independent consistency check of the performance of the purification process.	

4.5.5 Page 11 Line 13 (WYETH)	Well characterised processes and robust, efficient downstream purification steps developed for removal of leached Protein A should allow for a validation approach to residual Protein A level assessment. Validation approach for residual Protein A is indicated as acceptable in section 4.3.1. General considerations (line 1 page 7). Text expansion is proposed. Added text: "*A validation approach might be considered if supported by sufficient process characterization and validation data*."	
4.5.1 Page 10 Line 13 (PDA)	"Potency" should be replaced with "antigen binding" which should not necessarily be equated with potency. Replace "Potency" with "antigen binding"	*Text regarding potency assay reworded, to acknowledge that in situation where biological activity in clinical situation is only dependent on binding properties, binding assay may be deemed acceptable. However, when effector functions are relevant for clinical activity, other approaches should be considered.* *See adopted document for details.*
4.5.2 Page 10 Line 20 (MEDIMMUNE)	If the mode of action of the antibody is via binding or neutralisation, then a biological assay may not always be required. Revised text: "The potency/biological activity of the monoclonal antibody should be established by means of a suitable assay e.g. an assay that measures binding for an antibody whose mode of action is binding/neutralization."	
4.5.2 Page 10 Lines 20-21 (EFPIA/EBE)	Type of comment: minor While we agree it is preferable to establish a link to a clinical relevant parameter, EFPIA/EBE suggest additional explanations to clarify the intent of this sentence. Modify lines 20-21 as suggested: "~~The potency/biological activity of the monoclonal antibody should be established by a biological assay. It is preferable to establish a link to a clinical relevant parameter~~. *"Based on known biological mechanism of the antigen in vitro, a link to the in vivo mechanism of therapeutic action may be made."*	

4.5.2 Page 10 Line 21 (PDA)	While we agree it is preferable to establish a link to a clinical relevant parameter, this is not always possible. We suggest that including additional guidance as proposed would be useful. There should be *in vitro* tests applied that demonstrate the relevant mechanism of action to the therapeutic from *in vitro* clinical studies. Revise as follows: *"The potency assay should be linked to the known mechanism of action of the therapeutic from in vitro studies that take into account the known functional mechanism of action. If possible, it should be by a biological assay linked to clinically relevant parameters."*	
4.5.2 Page 10 Lines 23-24 (EFPIA/EBE) (PDA)	Type of comment: minor Provide additional wording for clarification insert "to the target" after the words "measures binding". Modify sentence as follows: "For binding/neutralizing antibodies, a potency assay that measures binding ***to the target*** (eg. an immunoassay in an ELISA format) may be deemed appropriate."	
4.5.2 Page 10 Lines 27-30 (EFPIA/EBE)	Type of comment: minor It is recommended to add additional examples of effector function. Modify sentence as follows: "A combination of two separate methods, one measuring the specificity and one giving an indication of an effector function (for example complement activation ***or C1q binding, or Fc gamma receptor binding)***, would be acceptable if a cell-based assay is not feasible or if the combination of two methods gives more precise results."	
4.5.2 Page 10 Lines 32-33 (MEDIMMUNE)	Expand this section. Description implies it is only effector functions such as complement binding and activation, etc. that may not be detected by a binding ELISA – this is not necessarily the only reason. Revised text: "… because there are product attributes that may not affect binding to target (such as fragmentation and glycosylation) but may affect further signalling or receptor expression."	

4.5.2 Page 10 Lines 34-35 (EFPIA/EBE)	Type of comment: minor The description of specific activity given (biological activity per unit of mass) differs from the description given under paragraph 4.4.2. EFPIA/EBE recommends further clarification to define specific activity. Modify as suggested: "Specific activity (biological activity per ~~unit of~~ mass ***or potency relative to reference standard***) is of considerable value to demonstrate consistency of production."	
4.5.3 Page 10 Lines 38-47 (EFPIA/EBE)	Type of comment: major While the glycan structures present should be as fully characterized as technologically feasible, appropriate specification setting for glycosylation is best achieved by considering the biological relevance of glycosylation for antibody bioactivity, safety, pharmacokinetics and immunogenicity. The focus of specification setting for glycosylation should therefore be on the testing of those quality attributes relevant for safety and efficacy; for example, in the case of antibodies which elicit significant effector function, core fucosylation is known to be an important parameter and therefore the fucosylation level should be controlled by a specification. In addition, specification setting for glycosylation parameters should incorporate appropriate use of risk assessments and linking of critical quality attributes for setting of specifications. Specifications for glycan structures need not be set solely to demonstrate process consistency, which can be assured by means other than by end product testing. For example, process consistency can be demonstrated throughout the product development history by appropriate comparability assessments and by evaluation of consistency through process characterization and process validation. It should be noted that specifications which are set to assure safety and efficacy can and should serve as process consistency checks. Replace lines 38-47 with this re-write: *"The effects of the Fc glycosylation on the biological properties and pharmacokinetics of the monoclonal antibody should be determined and limits established where relevant."*(*) (*): change also proposed by ROCHE (see below)	*Control of relevant glycosylation structures should be considered in the specification, taking into account the intended, as well as potential impact of this attribute on the biological activity in the context of the clinical situation.* *See adopted document for details.*

4.5.3 Page 10 Lines 38-47 (LONZA)	Release specifications for glycosylation have typically been set where a functional relationship has been demonstrated between glycosylation profiles and the mode of action or other parameters of clinical relevance. For those IgG molecules where there is no such correlation and/or the process validation studies have defined a stable and consistent glycosylation profile we do not see an absolute requirement to set a release specification for glycosylation. Revise text to specify that a glycosylation specification is recommended where a link to efficacy has been demonstrated or the process validation studies have indicated a requirement to monitor this parameter.	
4.5.3 Page 10 Lines 38-47 (ROCHE)	The G0/G1/G2 modifications do not affect clearance, nor do they modulate effector functions; galactosylation affects *in vitro* complement-mediated cell killing, but this is likely to be an assay artefact that diminishes as the C1q concentration approached physiological levels. The document does not discuss fucosylation directly, despite considerable literature demonstrating that this has a huge effect on *in vitro* ADCC. The need for a specification should be based on whether the glycan characteristics affect biological activity or clearance. Therefore, we recommend rewording this section: *"The effects of the Fc glycosylation on the biological properties and pharmacokinetics should be determined, and limits established where relevant. When a glycosylation specification is not appropriate, it may be useful to perform in-process testing and trending as a means of assessing manufacturing consistency*."	
4.5.3 Page 10 Line 44 (HAS)	"pharmacodynamic" is redundant here, it is covered by the first bullet point. Delete: "pharmacodynamic"	

4.5.3 Page 10 Lines 45-46 (MEDIMMUNE)	Based on the mechanism of action and the impact on pharmacokinetics, it should be determined whether a specification for glycosylation is relevant. Specifications for glycosylation should be based on comparison of the amounts of G0, G1 and G2 with each other. It may be difficult to quantify other minor glycoforms accurately or precisely. Revised text: "Based on the mechanism of action and the impact on pharmacokinetics, it should be determined whether a specification for glycosylation is relevant. If a specification for glycosylation is needed, the ranges for relative proportions of G0, G1 and/or G2 should be set based on the process capability."	
4.5.3 Page 10 Lines 45-46 (MSD)	Again, suggesting adding "f" or comment about the extent of fucosylation, since it is very important in effecter functions whether one has fucose or not and how much. Modify line 46 to read "… G2 relative to the total amount as well as the extent of fucosylation of these species) …".	
4.5.3 Page 10 Lines 45-47 (LUDGER)	Should include checks on levels of fucosylation and degree and types of sialylation. These checks are now easy to do routinely in biopharmaceutical QC labs with current glycoanalysis technology. Proposed rewording: *"Therefore, a specification for glycosylation (at least aimed at controlling the amount of G0, G1 and/or G2 relative to the total amount, levels of fucosylation and degree and type of sialylation) should always be set. In addition, such a specification will function as an independent check on manufacturing consistency."*	

4.5.3 Page 10 Lines 45-47 (PDA)	While specifications for glycoforms may be useful and necessary for comparability studies, they should not be necessary for lot release and shelf life studies UNLESS specific glycoforms have been shown to be required for proper function of the antibody and ONLY IF they are not reflected in the potency assay. Change the sentence to read: *"Therefore, if specific glycoforms are necessary for the proper function of the antibody and if the functional potency assay does not reflect the presence of the appropriate glycoform, a specification for glycosylation …should be set…"*	
4.6. COMPARABILITY		
Page no. + Line no.	**Comment and Rationale**	**Outcome**

4.6 Page 11 (EFPIA/EBE)	Type of comment: major Since comparability is closely linked to characterization and understanding the product and changes that may impact safety and efficacy, EBE recommends deletion of section 4.6 (Comparability) since the issue of comparability is not unique for monoclonal antibodies. Additionally, reference to ICH Q5E and the EMEA guidance, "Guideline on Comparability of Biotechnology-Derived Medicinal Products after a Change in the Manufacturing Process – Non-Clinical and Clinical Issues, 10/101695/2006" should address appropriately concerns regarding comparability.	*Section on comparability completely removed.*

4.6 Page 11 Line 35 (ROCHE)	We welcome addressing comparability in this guideline. The previous guideline (3AB4a), in its chapter 15.3, described the need of clinical studies in order to demonstrate product equivalence after manufacturing changes. Whereas this chapter now, for good reasons, has been replaced by reference to ICH Q5E guideline, the statement in the previous guideline *"When both monoclonal antibodies are demonstrated to have identical physico-chemical, biological and pharmacological characteristics, clinical studies performed with the former monoclonal antibody can be accepted. However, an essential prerequisite is that the production is based on the same MCB. Otherwise, clinical trials have to be carried out with the second form of antibody"* has been removed. We believe that, in the absence of specific guidelines on the comparability of monoclonal antibody products, the request for clinical data in case of change of the MCB which involves genetic manipulation (i.e., except if the change is based just on sub-cloning an existing MCB) should be maintained. This is mandatory especially because of the multi-functionality of biological activities of immunoglobulins which may be influenced in an unexpected way if the product is obtained from another cell, and where differences induced by changing the MCB may escape detection in in-vitro and preclinical assays. The design of the clinical trials should be decided on a case-by-case basis. Add reference to the draft EMEA draft guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process – non-clinical and clinical issues **(**EMEA/CHMP/BMWP/101695/06). After "ICH Q5E Guideline", add the sentence "*An essential prerequisite for acceptance of clinical data obtained with a pre-change monoclonal antibody product also for the post-change material is that the production is based on the same MCB. If a new MCB is created by a process involving genetic manipulation, clinical trials have to be carried out with the post-change form of the antibody. The design of these trials and the amount and type of clinical data to be presented has to be determined on a product-specific case-by-case base."*	

Page 11 Lines 38-41 (PDA) (ROCHE)	The statement "*monoclonal antibodies are fairly robust and changes in the structure may not affect pharmacological properties in vitro (for example binding to epitope, effector functions like activation of Fc receptors), although they may influence pharmacokinetic properties, efficacy and safety/immunogenicity in vivo*" is misleading and should be corrected. For example, it has recently been shown by S. Matsumiya et al. (J. Mol. Biol. 368, 767-779) that removal of the fucose residue from the oligosaccharides attached to a human IgG1 antibody results in a significant enhancement of ADCC (as assayed *in vitro*) whereas only subtle conformational alterations are detected. This means that even small structural changes (which may not be detected at a quick glance) may have significant impact on functional properties. If the aim of this sentence is to point out that *in vitro* studies alone may not be sufficient to detect changes of biological or functional relevance, wording should be clearer. Proposed rewording: *"In general, monoclonal antibodies are fairly robust and changes in the structure may not always affect pharmacological properties in vitro (for example binding to epitope, effector functions like activation of Fc receptors), although they may influence pharmacokinetic properties, efficacy and safety/immunogenicity in vivo."*	
Page 11 Lines 43-44 (PDA) (ROCHE)	Suggest adding "then the relevant aspects" as a qualifier. We may not want to look at ALL aspects of effector function each time, if only one aspect is likely to be influenced. Proposed rewording: *"If effector functions of the monoclonal antibody are part of the mechanism of action, then the relevant aspects of these should be fully re-assessed as part of a comparability exercise."*	

4.7. MONOCLONAL ANTIBODY-RELATED SUBSTANCES		
Page no. + Line no.	**Comment and Rationale**	**Outcome**

Section 4.7 Page 12 Line 1 (EFPIA/EBE)	Type of comment: critical Section Heading: "Monoclonal Antibody-Related Substances" The term "related substances" is used improperly throughout the document. This term should be reserved for the definition outlined in ICH Q6B. In some cases in the guideline, "antibody derived products" should be used or "antibody- related protein. Modify section heading using consistent definition for related proteins.	*Mab-related products have been removed from the core document, and will be addressed in annexes to the guideline as required.*
4.7 Page 12 Lines 4-8 (MEDIMMUNE)	Include conjugated fragments i.e. PEGylated Fabs Add to list: - conjugated monoclonal antibody fragments (including Fab	
4.7 Page 12 Line 5 (EFPIA/EBE)	Type of comment: minor Some further illustration by example regarding "fusion proteins" might be useful, since such products are currently authorised. Modify line 5 as follows: "-fusion proteins ***(eg fusion of soluble receptor domain with an Ig Fc region domain)***"	
4.7 Page 12 Line 16 (MEDIMMUNE)	Proteins in inclusion bodies must be solubilised before they can be refolded. Revised text: "If the antibody fragment is produced as an insoluble and inactive protein (inclusion bodies), solubilisation and refolding steps are required to recover the functional structure of the molecule."	
Section 4.7 Page 12 Lines 17-19 (EFPIA/EBE)	Type of comment: minor Misfolded and aggregated species need to be controlled, but may not be completely absent. These species need to be controlled and appropriately characterized and monitored. Modify sentence as follows: "In that case, special attention should be given to process validation in order to ensure correct refolding and disulphide bond formation as well as ~~the absence~~ ***sufficiently low levels*** of misfolded and/or aggregated species in the final product."	

Section 4.7 Page 12 Lines 25-28 (EFPIA/EBE)	Type of comment: major If it can be demonstrated during development that the chemically/biochemically modified antibody shows the same quality for a certain set of quality attributes both on the level of intermediate and final drug substance, it should be possible to define one set of specifications and thus to control the intermediate through the specifications of the final drug substance at the time of filing for marketing authorisation. Re-write lines 25-28 to: *"If monoclonal antibodies are modified by chemical or biochemical means after a primary cell culture (or fermentation) and purification stage, then the purified but unmodified antibody can be regarded as a critical intermediate. The quality dossier should include a full description of the production and control of the unmodified monoclonal antibody. The intermediate antibody may be controlled by appropriate specifications or may be controlled through release specifications of the final modified antibody based on data obtained during development."*	
4.7 Page 12 Lines 25-28 (MSD)	Assuming the intent is that monoclonal antibodies produced for use in antibody-related substances must be produced according to the same standards and with the same level of documentation as monoclonal antibodies employed directly as products, this intent should be stated unambiguously. (Otherwise, a different modification that unambiguously represents the intent should be made). Modify lines 27-28 to read: "Production of the monoclonal antibody intermediate product, and documentation of same, should be performed according to the same standards as for a monoclonal antibody being produced as an API. The quality dossier should include a full description....".	
4.7.1.2 Page 12 Lines 39-42 (MEDIMMUNE)	Consideration should be given to the source and quality of the enzymes used. Proposed new paragraph after line 42: "The source and quality of any enzymes and catalysts used in the digestion need to be appropriately documented, including details of any animal or human derived components."	

4.7.1.2 Page 12 Lines 41-42 (EFPIA/EBE)	Type of comment: minor Removal of enzymes should be validated to reach acceptable limits. Modify sentence as follows: "The removal of enzymes used for fragmentation should be validated ***to acceptable limits***."	
4.7.3 Page 13 Line 7 (EFPIA/EBE) (PDA) (ROCHE)	Type of comment: minor Add***:*** "-Conjugation with a toxin, another protein ***or a peptide.***"	
4.7.3 Page 13 Line 7 (HAS)	A rewording is proposed: "- Conjugation with a toxin or another active substance"	
4.7.3 Page 13 Line 8 (HAS)	The meaning of "targeting" is questionable in this context. It is the antibody that combines an active substance to the target structure. We propose a rewording: "- Conjugation with a chemical moiety to modify a functional property, half life, or for (radio)labelling"	
4.7.3 Page 13 Lines 11-12 (MEDIMMUNE)	Consideration should be given to the source and quality of the compound being conjugated to the antibody. Add this sentence: "Details of the chemical structure, quality and source of the moiety being conjugated to the antibody or fragment should be appropriately documented."	
4.7.4 Page 13 (EFPIA/EBE) (ROCHE)	Type of comment: minor In the case of bispecific antibodies, we suppose special attention should be given to their process validation and characterisation, considering their specific heterogeneity such as inappropriately assembled molecules, since a bispecific antibody molecule generally has two different heavy chains and two different light chains which may be assembled at random.	

4.7.5 Page 13 Lines 28-38 (EFPIA/EBE)	Clarification is required that this section does not apply to the radiolabelling of antibodies for the purpose of clinical investigational studies (e.g. ADME studies). Add sentence to end of line 29: *"These provisions do not apply to the radiolabelling of antibodies for the purpose of clinical investigational studies (e.g. ADME studies)."*	
4.8. MONOCLONAL ANTIBODIES USED AS REAGENTS		
Page no. + Line no.	**Comment and Rationale**	**Outcome**
4.8 Page 13 Line 1 (EFPIA/EBE)	Clarify that guideline only refers to antibodies used in purification and not for other purposes.	
4.8 Page 14 Lines 8-9 (PDA)	What is the rationale for the request of determination of the amino acid residues coupling to resin? At least for standard immobilisation techniques (like cyanogen bromid), this should not be required. Delete the sentence lines 8-9.	
DEFINITIONS		
Page no. + Line no.	**Comment and Rationale**	**Outcome**
Page 14 (PDA)	Add term Fc to the definition.	*Mab-related products have been removed from the core document, and will be addressed in annexes to the guideline as required.*
REFERENCES (scientific and/or legal)		
Page no. + Line no.	**Comment and Rationale**	**Outcome**

Page 15 (EFPIA/EBE) (ROCHE)	Add reference to the EMEA guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process - non-clinical and clinical issues **(**EMEA/CHMP/BMWP/101695/2006)	*References have been limited to documents in relation with Quality aspects.*