PDA Europe
Adalbertstr. 9
16548 Glienicke/ Berlin
Germany
Tel: + 49 33056 2377-0
Fax: + 49 33056 2377-77
Email: info-europe@pda.org
www.pda.org

Via Electronic Mail

30 November 2007

Dr. Pascal Venneugues
European Medicines Agency
7 Westferry Circus, Canary Wharf
London E14 4HB, UK
Email: pascal.venneugues@emea.europa.eu

Reference: Guideline on Production and Quality Control of Monoclonal Antibodies and Related Substances, 5 April 2007 (EMEA/CHMP/BWP/157653/2007)

Dear Dr. Venneugues,

PDA is pleased to provide comments to the EMEA on the subject guideline. Our comments were prepared by an expert committee of our members with practical experience in the field of monoclonal antibodies. The 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.)

Using these criteria, we have prepared detailed technical comments in the standard EMEA table format. As always, PDA focuses on scientific and technical issues and, where appropriate, offers specific recommendations to make the guidance more useful to all parties.

PDA would be pleased to meet with EMEA to discuss our comments. We would also be willing to attend and/or co-sponsor a public meeting to hear and understand the concerns of EMEA and to jointly work with EMEA on proposed alternative wording. If you have any questions please contact me, or my colleague Jim Lyda (lyda@pda.org), who did the staff work for our comments.

With very best regards,

Georg Roessling, Ph.D.
Senior VP, PDA Europe
Roessling@pda.org

cc: J. Lyda, R. Levy, R. Dana, Z. Kaufman

Attachment

European Medicines Agency

SUBMISSION OF COMMENTS
GUIDELINE ON PRODUCTION AND QUALITY CONTROL OF MONOCLONAL ANTIBODIES AND RELATED SUBSTANCES
EMEA/CHMP/BWP/157653/2007

COMMENTS FROM: Parenteral Drug Association (PDA), c/o James C. Lyda, lyda@pda.org

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.

Public

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

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 sialyted 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.

(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.

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

SPECIFIC COMMENTS ON TEXT		
GUIDELINE SECTION TITLE		
Line no[1]. + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)** **Underlined text = changed or new text**
p.4 Line 22	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."*
p.4, Lines 34-36	Clarify precursor document to this guidance. This is currently provided on the cover page, but should also be included in the body of the text.	Proposal: add line statement to read: *"This guideline replaces the guideline on "Production and quality control of monoclonal antibodies", EMEA 3AB4a, July 1995."*
p.5, Line 4-5	"*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). The activity of many monoclonal antibodies is also dependant on immune effector function such as antigen-dependent cellular cytotoxicity and complement-dependent cytotoxicity* *Add term Fc to the definitions on page 14*
p.5, Line 7-8	"*Based on their structure, monoclonal antibodies can be non-human, chimeric/humanized or human antibodies*": this statement is too simple. The list is not complete because monoclonal	*Add sentence* *"Furthermore, monoclonal antibodies can be modified concerning primary structure as well as glycosylation in order to modify their*

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

	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.	*immunogenicity or immune effector functions."*
p.5 Line 15	*"Human monoclonal antibodies are antibodies of entirely human sequence"*: This statement is scientifically not strictly correct because even "human" monoclonal antibodies used as therapeutic proteins, depending on how 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, e.g. *"human monoclonal antibodies are antibodies entirely derived from human germline immunoglobulin sequences"*
p.5, Line 16-20	Again, 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.	Add sentence "*Other approaches to reduce immunogenicity of animal monoclonal antibodies by in-silico or in-vitro techniques are emerging.*"

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

p.5, Line 34-40	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. Furthermore, please note the wording as stated in the second sentence of this paragraph, "*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 surrogate antibodies and therefore conflicts with ICH Q6. 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. 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.	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.* *"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".*
P6, Line 4 to line 23 (4.2.1)	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.	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.*

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

		The method of transformation needs to be assessed for potential safety concerns. *The use of continuous human B-lymphocyte as parental cell lines raise 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.*
P6, Line 25 to line 31 (4.2.2)	More detail should be provided regarding the requirements and documentation for the recombinant expression systems. The statement re 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 Please see the suggested text.	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*

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

	Change “obtained” to “obtain”	*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.’’*
p.6, 33-34	*“may be used to generate the monoclonal cell line”*	Change of wording: *“may be used to generate human Ig”*
Page 6 lines 40-43 and page 7 lines 1-15	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. 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. 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	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*

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

	possible; on the other hand, "consistency" has to be ensured not only with respect to heterogeneity, but regarding all aspects of the manufacturing process. Up to now no monograph for the monocyte activitation test is in place and therefore we would recommend deleting the reference to this test. After "non-endotoxin contaminants," seems to be phrased very generally. 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 as shown so that the safety requirements are met for parenteral preparations to ensure all aspects are addressed. Suggest delete the reference to Protein A, as this approach is not "an almost universal approach" to purification.	*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.*
page 7, Line 16 to Page 8, line 4.	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	Please refer to the following specific comments on chapter 4.3.2, pages 7 line 16 to page 8 line 4, to clarify to the reader how a manufacturing platform can be applied and used.

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

	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.	
p.7, Line 21	The word "*identical*" is misleading as this is not possible to achieve.	Change to "*comparable*"
p.7, Line 27-29	Strike the word "*optimised*" from this sentence. Additionally, the wording in this paragraph (lines 27-29) should be clarified. Suggested rewording is provided	"*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."*
p.7, Line 30-31	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.*
Page 7, Lines 31-38	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	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'*

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

	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.	*or if a unique approach is used.*
p.7, Line 39 - 43	This paragraph is very unclear. It is not clear if the discussion relates to 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.	Suggest the following text: *If a change is made to the platform process which will be implemented globally on several marketed products which were 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.*
Page 7 lines 45-48 4.3.2	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	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*

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

	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.	*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.*
p.8, Line 1-4	"Platform assays" are equally acceptable: *"… but it should have the same sensitivity and specificity for HCPs from different producer cell lines"*	Sentence should be rephrased: *"….but it should have the appropriate sensitivity and specificity HCPs from different producer cell lines"*
p.8, Lines 5 -16	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".*
p.8, Line 27-28	Reword to allow for use of appropriate analytical technology for confirming DNA sequence.	*The sequence should be deduced by DNA sequencing and confirmed experimentally by peptide mapping or other appropriate analytical technologies.*

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

p.9, Line 5-7	C-terminal Lys is not considered a product-related impurity. We recommend rewording the final sentence.	*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 extent of Lys-removal should be addressed.*
p.9, Lines 9-10	"*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 is also glycosylated within the variable domains; see for example L. Huang et al. (2006) Analyt. Biochem. 349, 197-207. This additional glycosylation site is assumed to arise from somatic mutation, but might also be introduced by in-vitro antibody generation technologies (such as phage display).	Change 1st sentence to: *"Typically, monoclonal antibodies have 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."* There are some reports on 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 (200), Biosci. Biotechnol. Biochem. 64 (11), 2298 - 2305
p.9, Lines 9-13	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.	Change lines 11 and 12 (last sentence) to read: "*All glycan structures present should be fully characterized, paying attention to those that affect biological activity of the monoclonal antibody."*
p.9, Lines 15-24	The immunological properties of the antibody should be described in a context dependent manner.	Reword the section lines 15 to 24 to read: *For all monoclonal antibodies the following characteristics should be described:*

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

		• *affinity and Kd;* • *antigenic specificity including the characterisation of the epitope that the antibody recognizes* • *the paratope (the part of the monoclonal antibody that recognises and binds to the epitope) should be identified* *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*
p.9, Line 18-19	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"*
p.9, Line 20	*"… 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;	This text should be deleted: *… the paratope (the part of the monoclonal antibody that recognises and binds the epitope) should be identified…"*
p.9, l. 22	This statement is redundant as this is described in section 4.4.3. We recommend deletion of "*the immuno-reactivity of the*	Delete: *"the immuno-reactivity of the antibody"*

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

	antibody" from this section	from this section.
Page 9 Line 22 4.4.2	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?	Not applicable
p.9, l. 23-24	We recommend deletion of, "*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	Delete *"the specific activity of the purified monoclonal antibody should be determined (units of activity/mass of product)"*
p.9, l. 25-34	Section 4.4.3. regarding the "Specificity and cross reactivity" The epitope determination as well as investigations on cross reactivity is not a matter for this guideline which addresses production and quality control. The section should be addressed in non-clinical guidelines. In the description given in this section it is not clear at which time point in development the investigations should be performed. Part of the preclinical investigations.	Delete all of the subject paragraph regarding *Specificity and cross reactivity"*
Page 9 Line 35 – 46 4.4.4	"(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	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 proteinaceuos particles including aggregates at release and during*

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

	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.	*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".*
p.10, Lines 11-12	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 to clarify the content: *Used test for identity testing should be specific and distinguish the product from any other product, e.g.IEF anti-idiotypic antibody or other appropriate method. If necessary a combination of methods should be used.*
Page 10 line 13, 4.5.1	"Potency" should be replaced with "antigen binding" which should not necessarily be equated with potency.	Replace "Potency" with "antigen binding"
Page 10 Line 21, 4.5.2	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 takes into account the known functional mechanism of action. If possible, it should be by a biological assay linked to clinically relevant parameters.*
Page 10	Insert "to the target" after the words "measures binding"	Insert *"to the target"* after the words "measures binding"

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

Line 24, 4.5.2		
Page 10, lines 45-47	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……*
Page 11 Line 10-17 4.5.5	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.'*
Page 11, Lines 10-17	**We recommend that Page 11 lines 10 to 33 are moved to the section 4.4.4 characterisation.** 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*	For moving to and addition to section 4.4.4: Suggest rewording 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*

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

	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*." Therefore, we believe that Section 4.5.5 should be reworded.	*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.*
p.11, Line 25-26	**We recommend that Page 11 lines 10 to 33 are moved to the section 4.4.4 characterisation.** Sentence should be reworded as follows:	*For IgG4 the relative percentage of half-antibody detected during molecule size distribution characterization should be addressed.*
Page 11 Lines 25-26, 4.5.6	**We recommend that Page 11 lines 10 to 33 are moved to the section 4.4.4 characterisation.** 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.*
p.11, Line 31-32	**We recommend that Page 11 lines 10 to 33 are moved to the section 4.4.4 characterisation.**	Change sentence to read: *Analytical ultracentrifugation and light scattering may be more*

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

	What about light scattering?	*suitable to isolate and characterise particulates.*
Page 11 Lines 29-33, 4.5.6	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".*
p.11, l. 38-41	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.	Change to read: *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.*
p.11,	Suggest adding "then the relevant aspects" as a qualifier. We may	Change to read*:*

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

Lines 43-44	not want to look at ALL aspects of effector function each time, if only one aspect is likely to be influenced.	*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.*
p.13, Line 7	- *"Conjugation with a toxin or another protein"*	Add*: "Conjugation with a toxin, another protein or a peptide"*
p. 14, Lines 8-9 Section 4.8	What is the rationale for the request of determination of the amino acid residues coupling to resin? At least for standard immobilisation techniques (like cyanogenbromid), this should not be required.	Delete the sentence line 8-9

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