

**WHO Guideline for the safe production and quality control of  
monoclonal antibodies for use in humans**

## NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed document – WHO *Guideline for the safe production and quality control of monoclonal antibodies for use in humans* – to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text **MUST** be received by **30 November 2021** in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Health Products Policy and Standards. Comments may also be submitted electronically to the Responsible Officer: Dr Richard Isbrucker at email: [isbruckerr@who.int](mailto:isbruckerr@who.int)

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide, second edition" (KMS/WHP/13.1).

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**1 Abbreviations**

2		
3	ADA	anti-drug antibodies
4	ADC	antibody-drug conjugate
5	ADCC	antibody-dependent cell-mediated cytotoxicity
6	CDC	complement-dependent cytotoxicity
7	CDR	complementarity-determining regions
8	CHO	Chinese hamster ovary
9	EBV	Epstein-Barr virus
10	ECBS	WHO Expert Committee on Biological Standardization
11	ELISA	enzyme-linked immunosorbent assay
12	EoPCB	end of production cell bank
13	GMP	good manufacturing practices
14	HCPs	host cell proteins
15	HLA	human leukocyte antigen
16	ICH	International Conference on Harmonization of Technical Requirements for
17		Registration of Pharmaceuticals for Human Use
18	LAL	<i>Limulus</i> amoebocyte lysate
19	LC-MS	liquid chromatography–mass spectrometry
20	mAb	monoclonal antibody
21	MAT	monocyte activation test
22	MCB	master cell bank
23	mRNA	messenger RNA
24	MSB	master seed bank
25	NCL	national control laboratory
26	NGS	next generation sequencing
27	NRA	national regulatory authority
28	PCR	polymerase chain reaction
29	PEG	polyethylene glycol
30	PPQ	process performance qualification
31	rDNA	recombinant DNA
32	rcDNA	residual cellular DNA
33	RTRT	real-time release testing
34	SBP	similar biotherapeutic product
35	SEC	size exclusion chromatography
36	SPF	specific-pathogen-free
37	SPR	surface plasmon resonance
38	TCID <sub>50</sub>	median tissue culture infective dose
39	TSE	transmissible spongiform encephalopathy
40	VLP	virus-like particle
41	WCB	working cell bank
42	WSB	working seed bank

43

## 1 Introduction

2  
3 The WHO *Guidelines for assuring the quality of monoclonal antibodies for use in humans* were  
4 adopted by the WHO Expert Committee on Biological Standardization (ECBS) at its forty-  
5 second meeting in October 1991 (1). Since that time there have been extensive technological  
6 advances in the manufacture and quality assurance of monoclonal antibodies (mAbs), most  
7 notably with the use of recombinant DNA (rDNA) and cloning technologies.

8  
9 In 2013 ECBS adopted the WHO *Guidelines on the quality, safety and efficacy of*  
10 *biotherapeutic protein products prepared by recombinant DNA technology* (2). Although  
11 guidance on the manufacture and quality control of mAbs is within the scope of that guideline,  
12 requests had been made to provide additional clarity and greater detail more specific to this  
13 subject, as well for guidance on mAbs manufactured from plant-based systems.

14  
15 The present guidelines have been developed through international consultation and are  
16 intended as a replacement of those in Annex 3 of Technical Report Series, No. 822 (1). As there  
17 have been significant advances in the types and varieties of mAbs and related antibody-like  
18 proteins, this guideline can be considered as applicable to those which are based on an antibody  
19 framework, including:

- 20
- 21 • mAbs of all isotypes, whether they are humanized, human, or chimeric, and regardless of
- 22 the intended therapeutic mechanism of action,
- 23 • antibody fragments, such as single-chain variable fragments (scFv's) and antigen-binding
- 24 fragments (Fab),
- 25 • single domain antibodies (nanobodies)
- 26 • bispecific or multispecific antibodies,
- 27 • mAbs or related antibody proteins which have been chemically modified, such as through
- 28 their conjugation to polyethylene glycol (PEG) or an active drug substance,
- 29 • multiple mAb substances pooled within a final product (“antibody cocktail”).
- 30

31 **For the purposes of this guideline, the term “monoclonal antibody” or “mAb” is**  
32 **used to encompass the breadth of the substances and products represented above unless**  
33 **otherwise stated.** Readers are also encouraged to consult with the WHO *Guidelines on*  
34 *evaluation of monoclonal antibodies as similar biotherapeutic products (SBPs)* for additional  
35 guidance on characterization and quality assessment for relevant products (3).  
36

37 It should be noted that this guideline is not applicable to nucleic acid-based platforms  
38 which use a vector or similar technology for the delivery of the genetic sequence that would  
39 encode for antibody production in vivo following their administration. The manufacture and  
40 quality assurance of such products is very different from those of mAbs. Although antibody  
41 mimetic proteins based on non-immunoglobulin scaffolds (e.g. DARPins, affimers, and  
42 anticalins) have some similar manufacturing and quality assurance processes as described in this  
43 guideline, they may also have unique regulatory considerations which are outside of the scope of  
44 this document. Therefore, manufacturers of such products are encouraged to refer instead to the  
45 above-noted guideline regarding biotherapeutics prepared by recombinant DNA technology (2).  
46

1 Although most mAb products are intended for parenteral administration, some are under  
2 development for their topical application, or to be administered intra-nasally, inhaled, or to be  
3 consumed orally. The manufacture and control of mAbs should be the same regardless of the  
4 intended route of administration; however, some specifications (such as endotoxin, bioburden, or  
5 purity) may not require the same levels of stringency as parenterally-administered products.  
6 Similarly, the selection of excipients may include those which are not suitable for parenteral  
7 administration.

8  
9 This guideline includes the production of mAbs and related proteins in animal systems,  
10 such as from mouse ascites. Despite providing the guidance on such products, WHO discourages  
11 the use of in vivo production methods, where possible, for the manufacture of these human  
12 therapeutic products.

## 13 14 15 **Terminology**

16  
17 The definitions given below apply to the terms as used in these WHO guidelines. These terms  
18 may have different meanings in other contexts.

19  
20 ***Adventitious agents:*** Contaminating microorganisms of cell cultures or ascitic fluids:  
21 including bacteria, fungi, mycoplasmas and viruses.

22  
23 ***Antibody fragments:*** Proteins which are short regions, or sections, of antibody  
24 molecules. These are usually antibody-binding fragments (Fab), single chain variable fragments  
25 (scFv), or single domain antibodies (e.g. nanobodies).

26  
27 ***Bispecific or multispecific antibodies:*** A single mAb in which each binding domain  
28 recognizes different epitopes of the same antigen or different antigens.

29  
30 ***Contaminants:*** Materials introduced to the substance and/or product which are not  
31 intended as part of the manufacturing process (e.g. adventitious agents, microbial contaminants,  
32 endotoxin).

33  
34 ***Drug product:*** A pharmaceutical product type in a defined container closure system that  
35 contains a drug substance, generally in association with excipients.

36  
37 ***Drug substance:*** The active pharmaceutical ingredient and associated molecules that may  
38 be subsequently formulated, with excipients, to produce the drug product.

39  
40 ***Impurities:*** Agents present in the substance or product which are either product-related  
41 (e.g. mAb molecular variants, aggregates, fragments) or process-related (e.g. reagents, media  
42 components, host cell proteins, leachates) and not considered the active ingredient.

43  
44 ***Intermediate:*** A material produced during steps of the processing of an active  
45 pharmaceutical ingredient or drug substance that undergoes further molecular change or  
46 purification before it becomes an active pharmaceutical ingredient or drug substance.

1  
2 ***MAb cocktail:*** A product formulated with two or more mAbs, mAb conjugates and/or  
3 mAb fragments which each recognize different epitopes or antigens. These may also be referred  
4 to as antibody mixtures, pooled antibodies or oligoclonal products.

5  
6 ***Master cell bank (MCB):*** An aliquot of a single pool of cells which generally has been  
7 prepared from the selected cell clone under defined conditions, dispensed into multiple  
8 containers and stored under defined conditions.

9  
10 ***Master Seed Bank (MSB):*** A seed of a selected plant from which all future production  
11 will be derived, either directly, or via a Working Seed Bank.

12  
13 ***Original cell line:*** The cell line produced or acquired by the manufacturer, on which the  
14 production of the MCB is based. The history of the original cell line (including details of parent  
15 cells, immunogens and fusion or immortalization methods) should be recorded whenever  
16 available.

17  
18 ***Recombinant DNA technology:*** Technology that joins together (i.e. recombines) DNA  
19 segments from two or more different DNA molecules that are inserted into a host organism to  
20 produce new genetic combinations. It is also referred to as gene manipulation or genetic  
21 engineering because the original gene is artificially altered and changed. These new genes, when  
22 inserted into the expression system, form the basis for the production of rDNA-derived protein(s)  
23 (2).

24  
25 ***Source material/starting material:*** Any substance of a defined quality used in the  
26 production of a biological product, excluding packaging materials.

27  
28 ***Working cell bank (WCB):*** The working cell bank is prepared from aliquots of a  
29 homogeneous suspension of a cell obtained from culturing the MCB under defined culture  
30 conditions.

31  
32 ***Working Seed Bank (WSB):*** A cell bank derived by propagation of cells from a WSB  
33 under defined conditions and used to initiate production cell cultures on a lot-by-lot basis

## 34 35 36 **General and Regulatory Considerations**

37  
38 Monoclonal antibodies (mAbs) are immunoglobulins, derived from a monoclonal cell line, that  
39 have a defined specificity. Their immunological activities are characterized by binding  
40 specifically to a ligand or antigen and may depend on other effector functions. Encompassing a  
41 wide range of clinical indications, they represent a large class of therapeutic biologicals that  
42 continue to transform modern medicine. In recent years, mAbs have dominated the  
43 biotherapeutics market and at the time of writing this guideline there were hundreds of novel  
44 mAbs and mAb-like proteins in clinical development (4). The success of therapeutic mAbs can  
45 largely be attributed to their specificity and the technological advances that have driven their  
46 development. With multiple functional domains within a single molecule, monoclonal antibodies

1 are, however, structurally and functionally complex proteins. This has implications for their  
2 production and quality control, and hence the way in which they are regulated.

### 3 4 **Antibody development**

5  
6 Historically, murine hybridoma technology, developed in the 1970s, paved the way for modern  
7 approaches to mAb discovery and continues to be used as the basis of generating chimeric and  
8 humanized mAbs by recombinant DNA methods. The development of phage display techniques  
9 in the 1990s provided a powerful approach to screening for peptides or antibody fragments (scFv  
10 or Fab) specific for therapeutic targets (5). Phage display technology has also been used to  
11 emulate antibody maturation, combining site-directed mutagenesis of CDR sequences with  
12 iterative cycles of affinity selection (6). Transgenic mice expressing only human  
13 immunoglobulin genes also provide an effective alternative for the identification of fully  
14 humanized antibodies. More recently, bispecific antibodies with novel functions have been  
15 engineered by linking antigen binding domains, such as scFvs or Fabs, with different  
16 specificities within the same antibody molecule. In addition, innovative products have been  
17 developed in which mAbs or antibody fragments have been conjugated to small molecule drugs,  
18 capitalising on their specificity to target drugs to particular sites or tissue.

19  
20 On-going improvements in antibody engineering combined with a greater knowledge of  
21 their immunomodulatory properties continues to give rise to new and improved products for the  
22 treatment of an increasing list of human diseases, each with specific target antigen(s) and  
23 mechanisms of action. Regardless of the process behind the development of the drug substance,  
24 the structure of the mAb is critical to the immunological and effector properties of the product.  
25 Regulatory assessment should be based on careful consideration of the rationale for the  
26 suitability of the mAb for its intended indication including the choice of its specific target(s), the  
27 affinity of the antibody for that target, and its mode of action. This requires a thorough  
28 understanding of the role of the target in the development of disease and the way in which the  
29 mAb exerts its biological effect; for example, blocking the binding of a ligand or infectious agent  
30 to a receptor, or mediating cytotoxicity via its Fc region.

31  
32 To ensure the safety and the efficacy of the product, the risk of it eliciting antibody  
33 responses in patients should be carefully considered, particularly if the structure or post-  
34 translational modification of the mAb differ from natural human immunoglobulin. Similarly,  
35 care should be taken to ensure the product does not induce hypersensitivity, autoimmunity or  
36 other adverse reaction in the recipient.

37  
38 The technology that allows a manufacturer to rapidly develop and produce various mAb  
39 products, each recognizing different epitopes or antigens but all based on common scaffold  
40 structures, poses unique issues that should be considered in their regulation. Experience gained  
41 from the development of one product can be applied in the development of another. This can,  
42 however, also lead to regulatory issues of similarity between products and whether they are  
43 considered as similar biotherapeutic products. In such cases, the reader is referred to the WHO  
44 *Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products (SBPs)*  
45 (3).



## 1 **Cell substrate and mAb production**

2  
3 The cell line chosen for mAb production must be stable in culture and produce a high titre of  
4 mAb that is correctly folded and glycosylated, but not aggregated. For these reasons, mammalian  
5 cells such as CHO and NS0 cells are currently most commonly used for the commercial  
6 production of humanized mAbs. Although expression systems based on plant, insect and  
7 microbial cells have also been developed, each presents its own challenges. Microbial production  
8 systems based on bacterial or yeast cells are potentially cheaper, more reproducible and easier to  
9 validate than mammalian cell cultivation. Despite these advantages, microorganisms often fail to  
10 express correctly folded and post-translationally modified mAbs. The ease with which plants can  
11 be cultivated and their potential for high production yield also makes them an attractive  
12 alternative to mammalian cells for the production of recombinant proteins. However, N-  
13 glycosylation in plant cells differ from mammalian cells and could have an impact on the  
14 stability, folding and biological activity of the recombinant mAb. Furthermore, the production  
15 process may require additional extraction steps to remove toxic alkaloids commonly found in  
16 plant material.

17  
18 The rationale for the choice of cell substrate should be considered in terms of its ability to  
19 produce biologically active protein of the desired quality. If the cell substrate is genetically  
20 engineered, the expression system should be described in accordance with the relevant WHO and  
21 ICH guidance. If cell fusion or transformation are used to immortalize B-lymphocytes for use in  
22 mAb production, the safety of the approach should be carefully considered, and where human B-  
23 lymphocytes are the parental cell line, careful consideration should be given to the possibility of  
24 contamination with defective prion or other pathogenic adventitious agents (see section A.4.2).

25  
26 The culture medium and growing conditions have a direct impact on cell growth and the  
27 amount of mAb produced. Mammalian cell culture media are inherently complex and  
28 traditionally have included animal sera in their composition to meet the exacting nutritional  
29 requirements of the cells. To reduce the risk from adventitious agents, such as the prion  
30 responsible for bovine spongiform encephalitis (BSE), well-defined media have been developed  
31 that are free from animal material, which are suitable for a range of cell substrates including  
32 CHO and NS0 cells. The development of defined production media has several advantages:  
33 improving consistency between production lots, providing greater control over production, and  
34 facilitating downstream purification and quality control processes. Mammalian cell culture  
35 conditions should be well defined and monitored in terms of temperature, pH, dissolved oxygen  
36 and carbon dioxide. Different culture conditions may be used to favour cell growth or mAb  
37 production and a two-phased approach can be employed, first to reach a required cell density and  
38 then to direct metabolism toward mAb production. Three types of production process have been  
39 used to culture cells for mAb production: batch production, continuous production using  
40 chemostat or perfusion cultures, and fed-batch processes. Fed-batch processes are currently the  
41 most commonly used on a commercial scale because of their high yield and advantages of being  
42 simple to scale up, control and validate.

## 43 **Downstream processing**

1 Following the production of the cell culture fluid, the mAb is recovered using a process that  
2 should consistently deliver a product suitable for human use. The first step of this process  
3 involves the removal of cells and cell debris. This initial purification is typically achieved using a  
4 combination of centrifugation, depth filtration and sterile filtration. Most mAb purification  
5 processes then involve protein A- or protein G-based chromatography. In a single step this  
6 results in a relatively pure product from which relatively small amounts of process and product  
7 related impurities are removed by subsequent rounds of chromatography. Finally, the product is  
8 concentrated and diafiltered into formulation buffer. The details of the downstream process are  
9 specific to each product and manufacturer. Consideration should be given to the source of  
10 protein A or protein G and its method of preparation to ensure a low risk of contamination with  
11 adventitious agents.

12  
13 Because of the structural similarity amongst mAb products and the technological  
14 experience of the manufacturer, it may be possible to develop so called platform manufacturing  
15 processes. These might include the cell culture system, expression vectors, purification schemes  
16 and analytical methods. The quality, safety and efficacy experience gained from one product is  
17 likely to provide supporting insights into the quality, safety and efficacy of another mAb product,  
18 made using the same technology and process, but recognizing different antigens. Nevertheless,  
19 manufacturing processes developed based on a platform manufacturing approach should be  
20 validated for a specific commercial product and the site where its produced. Given that quality  
21 attributes are product specific, the control strategy will also be product specific and the  
22 appropriateness of analytical methods developed for other products using the same platform  
23 approach needs careful consideration.

## 24 25 **Quality by design**

26  
27 Quality by Design (QbD) is a systematic regulatory approach to product development that  
28 utilises the detailed knowledge of a product, the processes employed in its manufacture and the  
29 associated process controls to ensure consistent product quality, safety and efficacy. The  
30 underlying principles of this approach are set out in ICH guidelines Q8 – Q11 (7-10). The  
31 extensive knowledge of antibody structure and function, together with ever-increasing  
32 experience of biopharmaceutical protein manufacturing processes makes the application of QbD  
33 an attractive option in the development of innovative therapeutic mAbs.

34  
35 The quality control and assurance of mAb products is challenging, largely due to their  
36 nature as very large and highly complex proteins with significant post-translational modifications  
37 that may impact their stability, pharmacokinetics and dynamics, immune reactivity, safety  
38 and efficacy. As biotechnological products, mAbs are likely to contain process- and product-  
39 related impurities with the potential to affect the quality of the product. Culture conditions can  
40 greatly influence mAb structure and both the purification processes and any genetic, post-  
41 translational or chemical modifications further adds to the challenge of producing a product with  
42 consistent quality. In light of this, it is important for the manufacturer to identify critical quality  
43 attributes of mAb substances and products early in their development stage and understand the  
44 impact of process changes.

## 45 46 **Conjugation to polyethylene glycol and drugs**

1  
2 Pegylation, the attachment of repeating units of polyethylene glycol (PEG) to a protein, is often  
3 used to protect therapeutic proteins from proteolytic cleavage, renal filtration and neutralizing  
4 antibody responses, thereby improving their pharmacodynamic and pharmacokinetic properties.  
5 Despite its pharmacological advantages, the pegylation process has the potential to give rise to  
6 inconsistency in the final product. Therapeutic proteins are typically conjugated to PEG by  
7 incubation with a reactive PEG derivative. Thus, the consistent structure and quality of the final  
8 pegylated product depends on the impurities introduced, the molecular weight distribution of the  
9 polymer and the conjugation chemistry employed. These factors should be addressed by the  
10 careful validation and control of the pegylation process. In general, site-specific conjugation  
11 technologies result in a more homogeneous product and offer better batch consistency than  
12 conjugation at random sites.

13  
14 The ability of mAb-drug conjugates to target cytotoxic molecules to specific cell types  
15 offers considerable potential for the development of innovative biopharmaceuticals, especially in  
16 oncology because of their power to discriminate between diseased and healthy tissue. Early  
17 mAb-drug developments, which were heterogeneous mixtures conjugated at random sites, have  
18 generally proved to be pharmacologically inferior to homogeneous products synthesised using  
19 site-specific conjugation. Therefore, like the methods used in pegylation, the technology used to  
20 conjugate mAbs to small molecule drugs needs careful consideration to ensure precise drug  
21 loading and a well-defined, homogeneous product.

## 22 23 **Heterogeneity**

24  
25 Although, by definition, mAbs are characterised by a single amino acid sequence, they are  
26 subject to post-translational modifications as well as physicochemical transformations that arise  
27 during their production and storage. In practice, the drug substance and the drug product usually  
28 also include a low level of sequence variants that arise from the inherent errors normally  
29 occurring during transcription and translation. Heterogeneity is specific to the manufacturing  
30 process and its potential impact on the activity, efficacy, safety, and pharmacokinetic properties  
31 of a mAb product should be understood to be able to ensure batch-to-batch consistency. In  
32 addition, heterogeneity may affect both the long-term stability and the immunogenicity of a  
33 therapeutic mAb, though in general, modifications that are found in natural human antibodies are  
34 less likely to be immunogenic. The types of modification commonly associated with therapeutic  
35 mAbs include: N- and C-terminal modifications, glycosylation, glycation, disulphide bond  
36 formation and various other amino acid related modifications.

37  
38 N-terminal pyroglutamate is a common modification of natural IgG; however, relatively  
39 minor changes in manufacturing conditions (e.g. buffer composition, pH and temperature) can  
40 result in variable levels of N-terminal pyroglutamate in therapeutic mAb products (11). Another  
41 common N-terminal modification associated with mAbs, rather than natural IgG, is the  
42 incomplete removal of signal peptides resulting in mAbs with signal peptides of variable size  
43 contributing to heterogeneity in the mass of the product (12, 13).

44  
45 Generally, mAbs are synthesized with a C-terminal lysine on their heavy chain that is  
46 subsequently removed during mammalian cell culture by basic carboxypeptidase activity.

1 Incomplete removal of lysine results in a product with variable levels of C-terminal lysine.  
2 Although C-terminal lysine does not affect mAb structure, stability or pharmacokinetic  
3 properties, its presence has been reported to interfere with C1q binding and complement-  
4 dependent cytotoxicity (CDC) (14). As heterogeneity caused by C-terminal lysine affects both  
5 mass and charge, it can be detected by mass spectrometry, isoelectric focusing or ion-exchange  
6 chromatography (15). In contrast to natural human IgG, which have very low levels, C-terminal  
7 amidation has also been reported as a common modification contributing to the heterogeneity of  
8 recombinant IgG1 mAbs produced in CHO cells (16).

9  
10 Like natural IgG molecules, mAbs have a conserved N-glycosylation site in the Fc region  
11 of IgG, which has a strong influence on antibody conformation and where certain glycan  
12 structures impact Fc $\gamma$  receptor binding. Based on its primary structure, the Fab region may also  
13 contain N-linked oligosaccharides which, depending on their location, may affect antigen  
14 binding. Antibodies that are aglycosylated tend to be destabilized, have a propensity to aggregate  
15 and are reduced in receptor binding activity, which has implications for their effector functions  
16 and immunogenicity. Aglycosylation contributes to mAb heterogeneity at low levels.

17  
18 In therapeutic mAbs, glycosylation-related heterogeneity is primarily caused by  
19 galactosylation, fucosylation, and sialylation of the biantennary complex oligosaccharides,  
20 although the presence of other low abundance oligosaccharides also contributes. Galactosylation  
21 may result in minor conformational changes at the site of glycosylation. IgG depleted in  
22 galactose has been shown to have reduced affinity for C1q and increased binding to the  
23 mannose-binding lectin (17-19). Increased galactosylation results in increased binding to Fc $\gamma$ RIII  
24 receptors (20). The level of galactosylation has little or no impact on mAb stability. Fucosylation  
25 also has a minor impact on mAb structure; however, depletion of core-fucose has been  
26 associated with enhanced ADCC (21) and therefore warrants evaluation depending on the  
27 therapeutic mechanism of the antibody. Although sialylation of the conserved Fc glycosylation  
28 site is generally low, high levels of  $\alpha$ 2,6 linked sialic have been shown to have a negative effect  
29 on ADCC (22). High levels of sialic acid have been associated with mAbs containing a Fab  
30 glycosylation site, but this appears to have little effect on antigen binding. Unlike animals,  
31 humans are unable to make the sialic acid N-glycolylneuraminic acid (Neu5Gc), which is  
32 typically found in mAbs produced in murine cells and has been linked to immunogenicity in  
33 humans (23).

34  
35 Other types of oligosaccharides can also contribute to mAb heterogeneity. In contrast  
36 with Neu5Gc, N-acetyl-D-glucosamine (GlcNAc) is only found in humans and not in the  
37 mammalian cells used to produce therapeutic mAbs. It appears to enhance ADCC but this is  
38 difficult to distinguish from the associated reduction in core-fucose, which has a similar effect  
39 (24). High mannose oligosaccharides are also frequently observed in mAbs (25). They contribute  
40 to heterogeneity in terms of the mass of the mAb and its separation on protein A  
41 chromatography. Although high mannose rich glycoforms tend to be cleared rapidly from serum,  
42 they have enhanced Fc $\gamma$  receptor binding and ADCC activity. MAb with high mannose glycan  
43 also have reduce C1q binding activity.

44  
45 Glycation is the reaction between reducing sugars and the primary amines on the N-  
46 terminus or lysine side chains. It primarily occurs during antibody production because of the

1 sugars present in cell culture media but also to a lesser extent during storage or when  
2 administered in diluent containing sugars (26). Glycation causes heterogeneity in both molecular  
3 weight and charge, as well as increasing the propensity of the mAb to aggregate (27). The level  
4 of glycation of antibodies is generally low and usually has little or no biological effect; however,  
5 in some cases the glycation of lysine in the complementarity-determining regions (CDR) can  
6 affect antigen binding (28). The impact of this type of glycation can be assessed during  
7 development of the product. Glycation appears to have little or no effect on Fc-related effector  
8 functions.

9  
10 The well-established inter- and intra-chain disulphide bond arrangements between  
11 cysteine residues in IgG play a key role in the folding and structural stability of an antibody.  
12 Therefore, any heterogeneity arising from variation in the disulphide bonding pattern in  
13 recombinant mAbs warrants careful consideration because of its potential impact on antibody  
14 structure, stability and biological activity. A number of variants have been identified including:  
15 alternative disulphide linkages; free sulphhydryl groups; trisulphide bonding; formation of  
16 thioether and cysteine racemisation (25). Variants with non-classical disulphide bond  
17 arrangements, which arise from the formation of different interchain connections between  
18 cysteines in the Fab and hinge regions, have only been described in IgG2 and IgG4, and can  
19 occur in both recombinant mAbs and natural antibodies. While non-classical variants of IgG2  
20 differ in biological activity compared with their classical counterpart, evidence suggests non-  
21 classical variants of IgG4 only differ in stability (29). Free sulphhydryl groups result from the  
22 incomplete formation disulphide bonds in both recombinant and natural antibodies. They may  
23 also arise when an antibody contains an extra cysteine residue, typically in the CDR. The impact  
24 of free SH-groups on biological activity or stability will differ from one product to another and  
25 should therefore be assessed for a given therapeutic mAb. The formation of a trisulphide bond  
26 from the interaction of an existing disulphide bond with hydrogen sulphide occurs during  
27 production and can be controlled by adjusting the culture medium or removed by introducing a  
28 cysteine wash during protein A chromatography. There is no evidence that trisulphide bonds  
29 affect antigen binding or thermal stability. The decomposition of disulphide bonds back to  
30 cysteine residues, through a dehydroalanine and persulphide intermediate, followed by cross-  
31 linking of dehydroalanine and cysteine results in the formation of a non-reducible thioether bond.  
32 In certain circumstances this may affect the structure of the Fab fragment and hence its antigen  
33 binding properties. This reaction also accounts for the occurrence of D-cysteine residues in the  
34 disulphide bonds between heavy and light chains in both mAbs and natural human IgG (30).

35  
36 Modifications to amino acid side chains are a major cause of heterogeneity observed in  
37 antibodies. Deamidation of asparagine and glutamine residues can occur at any stage during  
38 production and storage, depending on the external environment (e.g. buffer composition, pH and  
39 temperature). Residues in the CDRs are particularly susceptible to deamidation because of their  
40 flexibility and exposure to the medium. Isomerization of aspartate has also been observed in the  
41 CDRs of mAbs and, like deamidation of asparagine and glutamine, may impact antigen binding  
42 and potency. As a reaction intermediate of both asparagine deamidation and aspartate  
43 isomerization, succinimide is frequently found in CDRs and has also been shown to reduce  
44 potency (31). In addition, oxidation of several amino acids has been observed at low levels in  
45 natural human antibodies (32). In recombinant mAbs, methionine oxidation is often observed  
46 and when it occurs at the conserved residues in the Fc region causes conformational changes that

1 negatively impact stability, aggregation, complement-dependent cytotoxicity, binding to neonatal  
2 Fc receptor and in vivo half-life (33-35). Tryptophan residues in CDRs are sensitive to oxidation  
3 and can have a negative impact on potency, stability and aggregation (36).

4  
5 Aggregation is a common problem that contributes to the overall heterogeneity of protein  
6 therapeutic products, potentially compromising the quality, safety, and efficacy of mAbs.  
7 Aggregation caused by a wide range of conditions may occur at any time during the  
8 manufacturing process or storage. The size and nature of the aggregate is typically dependent on  
9 the kind of stress that led to its formation. Aggregation can result in the loss of a mAbs  
10 therapeutic properties and reveal new epitopes, which induce unintended immunity to the  
11 aggregate or the production of anti-drug antibodies (ADA) in the patient. Given its potential  
12 pharmacological impact on therapeutic mAbs, aggregation warrants careful consideration during  
13 the production and control of the product on a case-by-case basis.

## 14 15 **Characterisation**

16  
17 Given the challenges associated with manufacturing a product with consistent quality, it is  
18 important to have a robust strategy to characterise the drug substance and product to ensure  
19 critical quality attributes are maintained, and the product meets specifications. This  
20 characterisation would be expected to include an assessment of physicochemical and  
21 immunological properties, biological activity, heterogeneity, and the level of product- and  
22 process-related impurities.

23  
24 Drug substance and product characterisation typically includes methods for the  
25 determination of primary and higher order structure. The amino acid sequence of the mAb can be  
26 deduced from its nucleotide sequence and confirmed by peptide mapping and mass spectrometry.  
27 Nucleotide sequences of master and working seeds are conveniently determined using high-  
28 throughput methods. This is likely to be adequate as far as batch and fed-batch processes are  
29 concerned but the potential for genetic drift during continuous processes should be considered.

30  
31 Generally, physicochemical techniques offer the necessary sensitivity for the analysis of  
32 antibody heterogeneity (Table 1). However, the risk of artefacts arising from certain sample  
33 preparation methods must be taken into account. Variability in the N- and C- terminal amino acid  
34 residues can be assessed using methods that detect their impact on the charge and mass of the  
35 mAb. As disulphide bonds between cysteine residues play a key role in antibody folding and  
36 structural stability, it is important to consider the presence of free sulphhydryl groups and integrity  
37 of disulphide bridges. In addition, because of the potential impact of glycosylation on antibody  
38 structure and function, the carbohydrate content and glycosylation profile should be determined,  
39 paying particular attention to the distribution of glycan structures and the level of mannosylation,  
40 galactosylation, fucosylation and sialylation.

41  
42 Immunological characterisation of the mAb typically includes binding assays to  
43 determine its specificity, affinity and avidity for the target epitope. Examples of analytical  
44 methods for evaluating binding include ELISA, surface plasmon resonance, bio-layer  
45 interferometry and isothermal titration calorimetry. The antigen used in these assays and its  
46 relevant epitope should be defined and characterised as far as possible. Numerous methods are

1 available for mapping epitopes, each with strengths and weaknesses that need careful  
 2 consideration when choosing the appropriate method for a particular product. For example,  
 3 methods that only map linear epitopes would not be ideal for characterising a mAb that exerts its  
 4 function by binding to a conformational epitope. Methods that are commonly used for mapping  
 5 epitopes include: structural approaches such as x-ray co-crystallography and cryogenic electron  
 6 microscopy (cryo-EM); physicochemical approaches such as hydrogen–deuterium exchange and  
 7 cross-linking-coupled mass spectrometry; and methods based on scanning, such as array-based  
 8 peptide scanning and mutagenesis-based methods. To ensure the safety of the product,  
 9 unintended reactivity towards human tissues which are not the intended target should also be  
 10 determined during product development and other immunological effector functions (e.g.  
 11 complement activation, ADCC and Fc receptor binding activity) should also be evaluated.

12  
 13 The ability of the product to cause the desired effect (i.e. its biological activity) is usually  
 14 characterised using appropriate *in vitro* assays. The specific mechanism of action of therapeutic  
 15 mAbs is usually highly species-specific and the interaction of the Fc region of humanised mAbs  
 16 with non-human Fc receptors may give misleading results, limiting the usefulness of *in vivo*  
 17 assays in the characterisation of biological activity. *In vitro* methods typically used to assess  
 18 biological activity include ADCC and CDC assays, as well as neutralisation assays in the case of  
 19 mAbs used in the treatment of infectious diseases.

20  
 21 As discussed earlier in this section, mAbs commonly exhibit diverse forms of  
 22 heterogeneity resulting in a product that consists of a complex mixture of molecules. This  
 23 mixture, or purity/impurity profile, should be characterised by a combination of complementary  
 24 methods examples of which are provided in Table 1.

25

Table 1. Summary of potential sources of heterogeneity in recombinant mAbs and methods of detection

Heterogeneity	Physicochemical change	Method of detection	Comments
Primary structure	Amino acid sequence variation	Deduced from nucleotide sequence	Controlled by whole genome or deep sequencing master and working cell banks
N- and C- terminal modifications	Mass and charge	Ion exchange chromatography Isoelectric focusing Capillary electrophoresis	Detection methods can be combined with mass spectrometry for detailed identification
Glycosylation	Mass and charge	N-glycan release by PNGase F followed by hydrophilic interaction chromatography or capillary electrophoresis  LC-MS	Detection by fluorescence or mass spectrometry, does not provide site-specific information.  Peptide level MS required for site-specific data
Glycation	Mass and charge	LC-MS	Peptide level MS required for site-specific data
Hydrogen bond modifications			
Alternative disulphide linkages	Charge	Peptide LC-MS under reducing and non-reducing conditions	
Free sulphhydryl groups	Mass, charge and hydrophobicity		
Trisulphide bonding	Mass and charge		
Formation of thioether	Mass		
Amino acid modifications			
Asn deamidation	Mass and charge	LC-MS	Deamidation can be an artefact of sample preparation for LC-MS.
Asp isomerisation	Charge and hydrophobicity	LC and peptide LC-MS	
Succinimide	Mass, charge and hydrophobicity	LC-MS	

Oxidation	Mass and hydrophobicity	Reverse phase chromatography, peptide LC-MS	
Aggregation and fragments	Mass, visible and subvisible particle formation	Size exclusion chromatography with multiangle light scattering Light obscuration Nanoparticle tracking Microflow imaging	Due to potentially wide size range may need multiple methods

1

2

### 3 **Special considerations**

4

### 5 **Manufacturing and validation during product development**

6

7 MAb production, purification and other downstream processes may undergo considerable  
8 optimization after the initial clinical batches are produced. However, the process and product  
9 characterization should ensure the comparability of the mAb product throughout its development  
10 programme. Some changes in product characteristics can be anticipated; for example, following  
11 improvements in purification methods or conjugation chemistry. All such changes should be  
12 identified and presented in clinical trial submissions or during an application for a product  
13 license and the implications of the change should be discussed. It is not expected that process  
14 consistency will be demonstrated during early clinical development, partly because insufficient  
15 batches will have been produced to allow for adequate process validation and also because the  
16 process is likely to be subject to further optimization. However, all available batch data  
17 (including qualitative and quantitative data) should be presented. The product must be  
18 demonstrated to be free from contaminants and sufficiently characterized to allow bridging to  
19 subsequent clinical material and the commercial product.

20

21 The expectation of how rigorously GMP is implemented at this stage varies amongst  
22 regulatory authorities and consultation with the NRA early in product development is  
23 recommended. In some jurisdictions process validation may be expected to address safety issues  
24 such as aseptic operations, sterile filtrations, cleaning validations, environmental control of  
25 facilities and validation of the process utilities such as heating, ventilation and air conditioning  
26 (HVAC) systems, and water for injection systems.

27

28 During later clinical stages and at licensing submission, the manufacturing process may  
29 be firmly established and process-specific validation completed by demonstrating that at least  
30 three consecutive full-scale commercial batches can be made that conform to predetermined  
31 criteria. Although a “Quality-by-Design” approach is not considered in detail within these WHO  
32 Guidelines, such an approach is suggested in the development of manufacturing processes for  
33 mAb products (37) provided that the principles discussed throughout this document are  
34 adequately addressed.

35

### 36 **Special considerations for analytical procedures and specifications**

37

38 Testing of mAb substance(s) and of the final mAb product, as well as in-process control testing,  
39 may be expected to confirm the product safety of batches used in early clinical trials. In this  
40 regard, the NRA may expect tests for bioburden/sterility, endotoxin and freedom from



1 adventitious agents are developed, validated and applied to each batch. Other tests may not be  
2 fully validated; however, even from an early clinical phase, assay verification should have been  
3 performed. This is likely to fall short of the full validation requirements detailed in ICH  
4 guideline Q2(R1) on the validation of analytical procedures (38), but should nevertheless give an  
5 indication that each method is fit for purpose.  
6

7 Tests for safety, quantity, potency, identity and purity are mandatory for any mAb  
8 product through its clinical development programme. Upper limits should be clearly established  
9 for the quantity of both product- and process-related impurities, taking safety considerations into  
10 account. A justification should be provided for the quality attributes included in the  
11 specifications and for the acceptance criteria for purity, impurities (including aggregates),  
12 quantity, potency and any other quality attributes that may be relevant to the mAb product  
13 performance. The justification should be based on relevant development data, the batches used in  
14 nonclinical and/or clinical studies, and data from stability studies.  
15

16 It is acknowledged that during early clinical development, the acceptance criteria may be  
17 wider than the final specification for a product intended for Phase III studies and for commercial  
18 mAb products. During the production of the batches intended for clinical trial use, not all  
19 attributes tested may have established specification ranges as there may have been an insufficient  
20 number of batches manufactured that would be required to set an acceptable range. Nor at this  
21 time would a clinically meaningful range always be known. However, as the clinical programme  
22 continues – and certainly by the time of initiation of Phase III trials – specification ranges should  
23 be set for each attribute. Data from licensed mAb products made using the same platform  
24 technology and manufacturing process may be used as a guide to establish some specifications,  
25 such as criteria for process-related impurities.  
26

27 Product characteristics that are not completely defined in the early stages of development,  
28 or for which the available data are too limited to establish relevant acceptance criteria, should  
29 also be recorded. Such product characteristics could be included in the specification without  
30 predefined acceptance limits. At the initial stages of development, testing may not be required to  
31 determine residual levels of process contaminants (except rcDNA and host cell proteins) if  
32 sufficient justification can be provided by theoretical calculation. However, data to confirm the  
33 calculations should be provided prior to the licensing application.  
34

35 For later-stage clinical trials, it is expected that all analytical procedures would be  
36 qualified and some NRAs may expect them to be validated according to the principles set out in  
37 ICH Q2(R1) (38). Specifications for each parameter should be justified by the process capability  
38 as well as by clinical suitability. If justified, following the manufacture of additional batches of  
39 product, the sponsor should commit to revise the specifications as data on process capability are  
40 accumulated.  
41

42 During a public health emergency, data on clinical suitability are likely to be limited and  
43 should be taken into account to the extent that they are available. Under such circumstances, data  
44 from related licensed mAb products manufactured using the same platform technology and  
45 manufacturing process should be considered.  
46

1

## 2 **International reference materials**

3

4 Biological reference standards are used in qualifying or validating test procedures to ensure  
5 uniformity in the designation of potency or activity of biological preparations. These are required  
6 to ensure lot to lot consistency of production and to minimize the systematic deviation of assays.  
7 The WHO *recommendations for the preparation, characterization and establishment of*  
8 *International Standards (IS) and other biological reference standards* describes the preparation  
9 of National Standards (39).

10

11 WHO international reference preparations are available which are intended to support  
12 bioassays for some biosimilar mAbs. Relevant international reference reagents may also be  
13 available from WHO custodian laboratories for use in some quality control assays (for example,  
14 antigens that may be used to coat binding plates in the performance of ELISA). A catalogue of  
15 available international reference materials is available on the WHO website.<sup>1</sup>

16

## 17 **Part A. Manufacturing recommendations**

18

### 19 **A.1 Definitions**

20

#### 21 **A.1.1 International name and proper name**

22

23 Monoclonal antibodies and mAb fragments have, in general, been assigned international  
24 nonproprietary names which are composed of a random prefix, infixes which suggest its target  
25 class and species or recombinant origin, and followed by the stem “-mab” (40). Modified mAbs,  
26 such as those conjugated to a toxin or polyethylene glycol (PEG), mAb fragments, or which are  
27 bivalent are also reflected in the naming structure. Due to the large number of mAbs developed  
28 during the past decades, the breadth of diseases they are intended to treat, as well as the  
29 technological advances in mAb design, the WHO International Nonproprietary Name Expert  
30 Group regularly revisits and revises the naming scheme as required.<sup>2</sup>

31

#### 32 **A.1.2 Descriptive definition**

33

34 A mAb is, in general, a full-length immunoglobulin consisting of the constant domain (Fc), and  
35 the antigen binding domain comprising the antibody binding fragment (Fab) and variable  
36 fragment (Fv). Although the majority of commercially available mAbs are of an IgG isotype,  
37 other isotypes are considered within the definition of a mAb. MAbs may be genetically altered,  
38 chimeric, humanized and/or fully human, and chemically modified following their purification.  
39 MAb fragments consist of a section, or combination of sections, of the mAb, usually the Fab or  
40 Fv or may be single domain antibodies (VH or VL domains).

41

42 MAbs are derived from the expansion in culture of a single clonal cell expressing an  
43 immunoglobulin with affinity to a unique epitope, or unique set of epitopes (e.g. bispecific

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<sup>1</sup> <https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/catalogue>

<sup>2</sup> <https://www.who.int/teams/health-product-and-policy-standards/inn/>

1 mAbs), and may be generated using a variety of methods such as hybridoma, phage display,  
2 humanized transgenic mouse technologies, single B cell cloning, or recombinant DNA  
3 technologies. MABs may be produced in cultured mammalian cells, such as CHO, SP2/0 or NSO  
4 cells, human cell lines such as PER-C6 or HEK, as well as in bacterial cells, yeast, fungi, plants  
5 or cultured plant cells. The preparation may be generated from cells or plants producing only  
6 mAb fragments or genetically altered mAbs. Following purification, the mAbs or mAb  
7 fragments can be further modified to alter their pharmacokinetic and/or pharmacodynamic  
8 profiles. Product formulations may also combine two or more mAbs and/or mAb fragments that  
9 each recognize different epitopes or antigens are referred to as mAb cocktails. Unless intended  
10 for non-parenteral administration, mAb preparations should be presented as a sterile aqueous  
11 solution or freeze-dried material and should meet the specifications within this document.

12  
13 Due to potential differences among similar mAbs or mAb fragments, a clear description and  
14 characterization of all active substance(s) and the final product must be provided to the NRA.  
15 This may include details such as structural characteristics, subunit details, antibody  
16 class/subclass, chemical modifications and conjugations, and amino acid sequence.

## 17 18 19 A.2 General manufacturing recommendations

20  
21 The general good manufacturing practices (GMP) provided in WHO *good manufacturing*  
22 *practices for pharmaceutical products: main principles* (41) and WHO *good manufacturing*  
23 *practices for biological products* (42) should be followed at establishments manufacturing mAb  
24 products intended for human use. These practices include the safe handling of all manufacturing  
25 reagents and organisms under appropriate containment conditions based on risk assessment and  
26 applicable national and local regulations (41-43).

27  
28 For mAbs produced in plants, GMP practices are not considered practical to the upstream  
29 processes (e.g. cultivation, harvesting and initial processing of plants). In place, a stringent  
30 quality system must be established and implemented prior to marketing authorization of plant-  
31 derived mAbs. Although the WHO *guidelines on good agricultural and collection practices*  
32 *(GACP) for medicinal plants* (44) provides some useful guidance on this aspect, its intent is for  
33 use with medicinal plants, such as those used in traditional medicines, and the guideline is  
34 considered insufficient in establishing a stringent quality system for transgenic plant production  
35 systems.

36  
37 Regardless of the manufacturing process, manufacturers must conduct a process  
38 performance qualification with at least three consecutive batches at commercial scale prior to  
39 marketing authorization. All such batches should meet their specifications for both the  
40 substance(s) and product. The manufacturing process must be shown to consistently yield the  
41 substance(s) and product of satisfactory quality as outlined in this WHO guideline. All assay  
42 procedures used for the quality control of any intermediates, substances and final product should  
43 be validated at the time of commercialization.

44  
45 All post-approval changes to source materials, manufacturing processes, reference  
46 standards, or quality control test methods should be validated prior to implementation. If changes

1 to the source materials or production process are made during the development program or  
2 following its marketing authorization, then pre- to post-change comparability studies of the  
3 substance and/or product must be conducted. These changes may require approval from the NRA  
4 prior to implementation (45-47). Reporting categories and requirements for manufacturing  
5 changes can be found in the WHO *Guidelines on procedures and data requirements for changes*  
6 *to approved biotherapeutic products* (45).

7  
8 The development and use of transgenic (genetically modified) plants for the production  
9 of mAbs must conform to national and/or regional regulations and guidelines concerning their  
10 growth and use. When the bioengineered plant is from a species that is also used for food or feed,  
11 appropriate containment measures must be in place to ensure that there is no inadvertent mixing  
12 of the transgenic plant material with plants or plant material intended for food or feed use.  
13 Appropriate tests should be available that can detect the presence of the genetic insert and/or the  
14 product in the agricultural community. Proper environmental risk assessments must be conducted  
15 prior to growing transgenic plants in contained environments and their introduction to open  
16 fields. Additional resources and training on considerations surrounding the use of transgenic  
17 plants can be found in the Biosafety Resource Book developed by the Food and Agricultural  
18 Organization (FAO) of the United Nations (48).

### 21 A.3 Reference preparations

22  
23 In-house and secondary reference preparations should be established and maintained as  
24 described in the WHO *Recommendations for the preparation, characterization and*  
25 *establishment of International Standards (IS) and other biological reference standards* (39) as  
26 per the principles outlined in the WHO *Manual for the establishment of national and other*  
27 *secondary standards for vaccines* (49). If an international standard or reference material is not  
28 available, an aliquot of a lot that met the specifications in place at the time of the product release  
29 shall be used as in-house reference material. The criteria for establishing manufacturers'  
30 reference materials and their specifications should be approved by the NRA.

31  
32 All reference standards must be assessed for their suitability for their intended purpose.  
33 Those reference standards to be used in quantitative methods (for example, in the determination  
34 of potency), require a rigorous assessment to establish their true value. The number of  
35 determinations used to set the value must be statistically justified and take into consideration the  
36 inherent intra- and inter-assay variability of the method. The evaluation of all reference materials  
37 should include tests to assess the appearance, pH, protein concentration, identity, purity, and  
38 activity or potency. Biological reference materials should also be fully characterized to include  
39 any relevant structural characteristics as well as post-translational and chemical modifications.  
40 All reference materials must meet their specifications at the time of use.

41  
42 A two-tiered reference standard system consisting of a primary reference standard and a  
43 working reference standard is strongly recommended. The primary reference standard should be  
44 used for the requalification of each working reference standard batch, as well as to qualify future  
45 primary reference standards. The reference standards are evaluated using the same test methods  
46 as the bulk specification, or a subset of these methods, as well as any relevant characterization

1 methods. The primary, secondary and tertiary structures, as well as protein concentration, purity,  
2 quality, and potency of subsequent reference standards must be confirmed against the established  
3 specifications of the prior reference standards. Further characterization may include post-  
4 translational modifications, thermal stability and isoelectric points. If possible, it is  
5 recommended to establish the initial primary reference standard and working reference standard  
6 at the same time and from the same lot.

7  
8 Reference standards should be requalified on a regular basis, usually annually. If using a  
9 two-tiered system, only the primary reference standard may need to be requalified. For the  
10 requalification, quality attributes capable of assessing potential changes that may influence  
11 product quality should be selected. If the reference standard does not meet its specifications, it  
12 should be promptly replaced. The qualification program should be put in place prospectively.

13 In cases where a mAb preparation has a short validity period (e.g. radiolabeled mAbs),  
14 the reference material may comprise the unlabeled product and/or a product with non-radioactive  
15 label/conjugate.

16  
17 All reference preparations should be stored under conditions that maintain its stability for  
18 use in assays of subsequent lots. Shelf life and storage conditions for reference preparations  
19 should be determined based on its stability data.

#### 20 21 22 A.4 Control of source materials

23  
24 A consultation with the NRA is recommended for novel expression systems not discussed in this  
25 guidance document.

26  
27 All materials used in the manufacture of the drug substance and drug product should be  
28 listed in a submission including, but not limited to, any media components, enzymes, solvents,  
29 buffers, conjugation reaction reagents, and resins used in purification columns. The information  
30 on all raw materials should include the source, control tests, specifications, and where in the  
31 manufacturing process it is used. For mAb conjugates, the quality control and characterization or  
32 testing of the linker molecule and the compound(s) to be conjugated to the mAb should also be  
33 considered prior to the conjugation process.

34  
35 Manufacturers are encouraged to avoid the use of materials of animal origin. However, if  
36 the culture medium does contain materials of animal origin, these should comply with the current  
37 WHO *Guidelines on transmissible spongiform encephalopathies in relation to biological and*  
38 *pharmaceutical products* (50). All materials of animal origin should be tested for contaminants  
39 and verified to be free of adventitious agents. The use of materials of animal origin should be  
40 discussed with and approved by the NRA. The culture medium used in the preparation of  
41 commercial product lots should also be free from substances likely to cause toxic or allergic  
42 reactions in humans. This may include, for example, some antibiotics, methotrexate, albumin,  
43 serum, or insulin.

44  
45 Manufacturers should take careful note that changing the cell line or cell type for  
46 production of mAbs after their development requires that comparability studies are done between

1 products derived from the old and the new expression system. The comparability studies required  
2 would be dependent on the development stage for the product and the extent of any anticipated  
3 post-translational changes that may occur in the new expressing host cell. Comparability studies  
4 might only require physico-chemical and in vitro biological analysis, although it may be  
5 necessary to conduct nonclinical and clinical bridging studies to demonstrate safety, efficacy and  
6 the bioequivalence of the mAb generated in the new host cell system.

7  
8 The immunological specificity of the mAb should be verified during development of the  
9 cell or plant production system. Testing should include the mAb capacity to react with the target  
10 antigen, its isotype and light-chain composition. Additional testing to verify the mAb identity is  
11 also recommended and could include Western blot, verification of the mRNA by polymerase  
12 chain reaction (PCR), glycosylation analysis, amino acid and/or peptide mapping analysis by  
13 mass spectrophotometry.

#### 14 15 **A.4.1 Generation of mAb expression systems using rDNA technology**

16  
17 MAbs manufactured via rDNA technology should be produced using reliable and continuous  
18 host cells or host plants. Details of the host cells or plants, including their origin, source and  
19 history, should be provided. All starting and source materials used in the growth and  
20 maintenance of the host cells should be adequately controlled.

21  
22 Various prokaryotic and eukaryotic expression systems are available which can be used  
23 in the production of mAbs. Common prokaryotic cell lines include *Escherichia coli* (*E. coli*) and  
24 *Pseudomonas putida* and are the system of choice for non-glycosylated mAbs and mAb  
25 fragments (51). Common eukaryotic systems include mammalian, yeast, fungal, and insect cell  
26 lines, as well as plants. At the time of writing this document, Chinese hamster ovary (CHO) cells  
27 were the most commonly used expression system for the production of mAbs although murine  
28 SP2/0 and human HEK293 cells also have a strong history of use.

##### 29 30 **A.4.1.1 Expression vector and host cell**

31  
32 The process for deriving the expression vector and selection of the host cell should be described  
33 in detail. The source and history of the host cell, as well as any prior genetic manipulation or  
34 engineering it may have undergone for its selection as a host should be included. Details should  
35 be provided on the vector, identity of any cloned gene, as well as the genetic elements and  
36 function of the component parts of the vector. Important component parts to note include its  
37 origins of replication, any promoters and antibiotic markers, as well as a restriction enzyme map  
38 that indicates the sites used in the development of the vector. The coding sequence for the  
39 expression vector should be understood and verified it is correctly incorporated into the host cell.

40  
41 Details of the transformation into host cells, the rationale for the selection of the cell  
42 clone used for production, a determination whether the vector remains extrachromosomal or  
43 integrated, and its copy number should all be reported. All of the measures used to promote and  
44 control the expression of the cloned gene should be described in detail.

##### 45 46 **A.4.1.2 Transgenic plants**

1  
2 The selected source plant should be capable of producing a consistent product when grown under  
3 its intended conditions in either a controlled environment or open field. As plants may produce  
4 secondary metabolites (e.g. toxins or other bioactive substances) in response to their growing  
5 environment, stressors, or genetic manipulation, it is crucial to understand which relevant  
6 secondary metabolites the plant is capable of generating to ensure the implementation of proper  
7 downstream testing and purification processes.

8  
9 Traceable documentation should be provided which includes details on the  
10 characterization of the recombinant DNA constructs or viral vectors, as well as any other genetic  
11 manipulations used to transfer genes into the plant. Stability of the gene expression system and  
12 its continuation through seeds or plant cuttings must also be clarified.

13  
14 Source materials with appropriate quality attributes for the production of mAbs in plants  
15 should be used. Each lot of source material should be assessed for the presence of foreign matter.  
16 Care should be taken to minimize contaminants (e.g., molds and other agents) that could lead to  
17 the inadvertent exposure of recipients to undesirable impurities or could affect product quality.

#### 18 **A.4.2 Generation of hybridomas for the production of mAbs**

19  
20  
21 Methods used for lymphocyte isolation, fusion of lymphocytes with myeloma cells,  
22 immortalization of lymphocytes, selection of hybridomas and screening of mAbs must be  
23 recorded.

##### 24 ***A.4.2.1 Material used for immunization***

25  
26  
27 The antigenic material, including any adjuvant, used for the generation of immune lymphocytes  
28 should be defined. If the immunogen is derived from a human source, relevant clinical data on  
29 the donor should be recorded.

##### 30 ***A.4.2.2 Immune parental cells***

31  
32  
33 Where possible, the source of the immune parental cells should be documented. For murine  
34 mAbs, information on the animal strain should be provided, including its specific-pathogen-free  
35 (SPF) status. Where possible, the animals used for immunization should be SPF.

36  
37 For human immune parental cells, all data relevant to possible viral infections of the  
38 human donors should be available. The donated samples of immune parental cells should be  
39 screened for potential viral contamination, and in accordance with national requirements for  
40 blood donations and use of blood products.

##### 41 ***A.4.2.3 Immortalization procedures***

42  
43  
44 For animal cells and animal-derived cell banks, reference should be made to the WHO  
45 *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of*  
46 *biological medicinal products and for the characterization of cell banks* (52). Where myeloma

1 cells are used, they should be fully described, including details of their source, origin, history,  
2 name, and characteristics, as well as the storage culture conditions used in their expansion prior  
3 to fusion. It is preferable to use immortalizing cells that do not synthesize immunoglobulins  
4 themselves.

5  
6 Human B lymphocytes are usually immortalized by infecting them with Epstein-Barr  
7 Virus (EBV); however, this procedure alone cannot always ensure stability, and subsequent  
8 fusion with a myeloma may be required. If EBV is used for immortalizing human B  
9 lymphocytes, its origin and characteristics should be clearly specified.

10  
11 Before being fused or immortalized, cell cultures should be tested for their sterility  
12 according to the WHO *Requirements for the sterility of biological substances* (53, 54) or using  
13 suitable methods approved by the NRA. All cells should be found negative for bacterial, fungal,  
14 viral and mycoplasma contamination. Any identified viral contamination (e.g. EBV) should be  
15 documented and the risk assessed for its control and demonstration of removal during  
16 downstream processing.

#### 17 18 **A.4.3 Animals used for mAb production**

19  
20 If animals are intended for the production of mAbs from their ascites, they must be from SPF-  
21 monitored colonies and shall be free from viruses for which there is evidence of capacity for  
22 infecting humans or primates. Both the animals and the cells injected into the animals should be  
23 tested for possible murine viruses using PCR or other nucleic acid amplification method. If  
24 animals are found to be contaminated with viruses for which there is no evidence of capacity for  
25 infecting humans or primates, the final product may be accepted only if the purification process  
26 has been demonstrated to eliminate the infecting virus(es).

#### 27 28 **A.4.4 Cell/seed bank system**

29  
30 The production of mAbs should be based on a cell/seed lot system consisting of a master and  
31 working banks. Cultures or plants derived from the working bank should have the same  
32 characteristics as the cultures or plants from which the master bank was derived. Information on  
33 the establishment, characterization and cloning of the original cell line used to establish the cell  
34 bank shall be provided. As described in the WHO *Recommendations for the evaluation of animal*  
35 *cell cultures as substrates for the manufacture of biological medicinal products and for the*  
36 *characterization of cell banks* (52), a single cell clone should be isolated for expansion into a cell  
37 bank regardless of the source of the cells.

38  
39 To speed the development of products to patients, particularly during emerging outbreaks  
40 and public health emergencies, the use of a stable cell pool in lieu of a clonally derived cell bank  
41 may be considered for early clinical batches. This strategy should be discussed with the NRA.  
42 High density CHO cell cultures using transient expression processes may also be used to speed  
43 the production and evaluation during emerging outbreaks.

44  
45 Once a pure culture is established, it should be subcultured for production into a master  
46 cell bank (MCB). The use of a MCB system will reduce the future risk of contamination or loss



1 of the pure culture. It is strongly recommended that a two-tiered cell banking system is used with  
2 working cell banks (WCB) being derived from the MCB. Although a one-tiered system  
3 containing only a MCB is acceptable, its use must be justified.

4  
5 During the product development program the production system should be demonstrated  
6 to yield a consistent mAb during growth of the cells. The cells at the limit of in vitro production  
7 (i.e. at finite passage) should be characterized to demonstrate this consistency and compared to  
8 that of cells from the MCB. The expression vector should also be analyzed to insure that the  
9 correct coding sequence is maintained through to the final passage.

10  
11 For transgenic plants, the stability of the transformant should be established. Once  
12 verified, a two-tiered master seed bank (MSB) and working seed bank (WSB) system should be  
13 employed.

14  
15 Details on the cell or seed banking system should be well documented and include  
16 information pertaining to cell bank plans, size, types of containers and closure systems,  
17 development of the cell bank(s), cryoprotectants, media used, culture or growth conditions, long  
18 term storage conditions, and evidence of stability of the expression system under those  
19 conditions. Long term stability monitoring plans for cell or seed banks should also be established  
20 and documented in marketing authorization applications. It is expected that all cell and seed  
21 banks be monitored for their viability, identity, purity, stability, and vector copy number in order  
22 to demonstrate and ensure their stability.

#### 23 24 *A.4.4.1 Control of master cell or seed banks*

25  
26 All cell banks, regardless of the cell type, should be tested early in the development programme  
27 to confirm their identity and purity as well as to establish the suitability of the cell system for the  
28 production of mAbs with consistent quality. The extent of the cell characterization during the  
29 development process can influence the type and degree of routine testing needed at later stages  
30 of manufacturing.

31  
32 All MCBs must be sterile and free from mycoplasma (if derived from a mammalian cell),  
33 contaminating microorganisms, and any adventitious agents. Bacterial cell banks must also be  
34 free from bacteriophages. For MSBs, the level of bioburden should be controlled.

35  
36 All master cell and seed banks must be verified for their purity, identity, demonstrated to  
37 contain the appropriate product-specific coding sequence, as well as ability to produce the  
38 correct mAb or mAb fragment.

#### 39 40 *A.4.4.1.1 Identity tests for substrates*

41  
42 The identity of all cell and seed banks must be confirmed. The selection of appropriate identity  
43 test methods will be influenced by the cell type, culture or growth conditions, available  
44 resources, and whether other cell cultures or plants are maintained in the same facility. Some  
45 acceptable methods include:

- 1 • phenotyping;
- 2 • isoenzyme analysis;
- 3 • karyotyping;
- 4 • HLA typing;
- 5 • gene sequencing or next-generation sequencing (NGS);
- 6 • short-tandem repeat (STR) analysis.

7  
8 Phenotyping methods, such as the observation of cell or plant morphology and growth  
9 curve analysis, can provide early feedback into the performance of a cell culture and identify  
10 when problems may be arising in the stability of a cell/seed bank during its storage. Isoenzyme  
11 analysis can identify the species of origin, but does not distinguish cross-contamination with cell  
12 lines from the same species. Additional methods should be considered when multiple cell lines  
13 derived from the same species are used within a facility. The selection of appropriate genetic  
14 analysis will largely depend on the type of cells or plants used in the manufacture of mAbs, the  
15 risk of cross-contamination with other cells, and the risk of genetic changes during storage or  
16 growth. Gene sequencing or NGS can range from a single gene analysis to whole genome  
17 sequencing; the latter is recommended for microbial cultures with verification against published  
18 reference genome sequences.

#### 19 20 *A.4.4.1.2 Tests for microbial contaminants*

21  
22 All cell banks should be tested for relevant bacterial, fungal and/or viral contaminants. An  
23 assessment of specific viruses and the families of viruses that may potentially contaminate the  
24 cell bank should be conducted to help in the selection of appropriate panels for testing.  
25 Mycoplasma contaminants should be tested in MCBs of mammalian cell origin. Appropriate  
26 tests for detecting mycoplasma include direct and indirect culture methods and PCR assays.  
27 Bacterial cell banks should be tested for bacteriophages, and a bacteriophage contamination  
28 protocol should be in place in the event of a contamination being detected.

29  
30 For plant seed banks, the level of bioburden should be controlled.

#### 31 32 **A.4.5 Control of working cell or seed banks**

33  
34 If the WCB/WSB is prepared in a manufacturing facility different from where the MCB/MSB  
35 was established, the full testing regime described in section A.4.3.1 should be conducted.  
36 However, if the production conditions have not changed and a replacement WCB/WSB is  
37 derived from the same MCB/MSB as that used for preparing the prior WCB/WSB for which the  
38 test results had been found satisfactory, then the tests described in A.4.3.1 and tests for absence  
39 of viral contaminants may be omitted.

40  
41 Tests of the culture or seed purity and identity should be performed on each WCB/WSB.

#### 42 43 44 **A.5 Control of mAb production**

45

1 The manufacturing process must be validated before licensing and include an evaluation of all  
2 process steps to ensure they can consistently yield a substance and product of adequate quality.  
3 An understanding of the heterogeneity of mAbs, as well as the impact of process changes on the  
4 heterogeneity profile of the substance is important to establish during the development program.  
5 As the field of analytical chemistry and technologies is advancing rapidly, only some of the more  
6 commonly used methods that may be used in the analysis of mAb structure, function and quality  
7 are mentioned in this document. The implementation of new or novel analytical technologies  
8 should be discussed with the NRA.

9  
10 While manufacturing details and safety issues may be different between the various  
11 expression systems, some general principles can be applied. Appropriate in-process testing  
12 should be selected which takes into consideration potential safety concerns of the expression  
13 system.

14  
15 Manufacturing steps should be taken to prevent or control contamination by viruses,  
16 bacteria, fungi, mycoplasma and transmissible spongiform encephalopathy (TSE). Production  
17 techniques should be used that have been demonstrated to minimize any impurities inherent from  
18 the production processes and prevent the introduction of contaminants external to the  
19 manufacturing process. Potential impurities and contaminants which should be considered  
20 include peptides and proteins which do not constitute the substance or drug products, host cell  
21 proteins (HCPs), DNA, endotoxin, non-endotoxin pyrogens, viruses, culture media constituents,  
22 elemental impurities, components that may leach from columns during purification steps,  
23 reagents from any conjugation process as well as substances which remain unconjugated.  
24 Emphasis should be on minimizing the risk of contamination from the environment or cross-  
25 contamination from other products and consider the operational and design features of the  
26 purification suite, HVAC and other support systems, equipment, transfers of any intermediates or  
27 substances, and movement of personnel.

## 28 29 **A.5.1 Production of drug substance**

### 30 31 *A.5.1.1 Production from cell cultures*

32  
33 Only cultures derived from a qualified cell bank shall be used for production. The use of  
34 chemically defined and serum-free growth media is preferred over media containing animal  
35 serum. If animal serum is included in the medium used for the production of cell cultures, it must  
36 be tested to show its freedom from bacteria, fungi, viruses, and mycoplasmas. Each batch of  
37 serum shall be of certified origin and, if bovine, shall come from herds certified by the  
38 appropriate authority to be free from TSE (50). Test results provided by the supplier of the serum  
39 may be sufficient if the tests are performed according to validated and well documented  
40 procedures. Similar control measures and testing procedures should be included for any animal-  
41 derived substance (e.g. porcine trypsin) which may be used in the production of mAbs.

42  
43 Appropriate in-process controls and monitoring programmes should be in place to ensure  
44 the production of consistent substance(s). The consistency of the growth of the production strains  
45 should be demonstrated by monitoring their growth rate, pH, pO<sub>2</sub> and the final yield of mAb  
46 substance; however, monitoring should not be limited to these parameters and should be selected

1 based on consideration of the cells and cell culture system used. Control tests may be done by  
2 real-time release testing (RTRT) or fixed point release.

3  
4 Samples from the culture system should be taken during and/or at the end of the  
5 production and examined for microbial contamination. If the cells are to be inactivated or lysed  
6 prior to purification, the samples should be taken before that step. The purity of the culture  
7 should be verified using suitable methods, such as inoculation into appropriate culture media. If  
8 contamination is found, the culture and any substance or product derived from it should be  
9 discarded.

10  
11 If an inactivation or cell lysis method is used, it should be monitored to ensure  
12 completeness using a validated test during routine manufacturing. If a chemical agent is used for  
13 cell inactivation or lysis, validated methods for its detection must be in place and residual levels  
14 should be controlled. The impact of the inactivation process on mAb heterogeneity should also  
15 be evaluated.

#### 16 ***A.5.1.2 Production in transgenic plants***

17  
18 For each process that is not intended to be sterile, extraneous bioburden should be controlled  
19 using procedures to minimize the introduction of potential contaminants and through in-process  
20 testing.  
21

22  
23 For greenhouse-grown material, the types of containers, soil mix composition, and  
24 greenhouse growth conditions can impact product quality. For field grown material, the previous  
25 uses of the land (e.g., agricultural and/or industrial use) can also affect product quality and  
26 should be documented. Specifications, acceptance criteria, and other limits should be established  
27 for the soil composition and potential soil contaminants that may affect production. In addition,  
28 agricultural methods utilized during crop growth, including specifications regarding the use of  
29 chemicals and limits on specific agricultural practices (e.g., the use of specified fertilizers,  
30 pesticides, or herbicides, and irrigation practices relative to a specified harvest time frame, etc.)  
31 should be in place. All pest-control measures implemented should be in accordance with national  
32 and/or regional agricultural requirements and best-practices.  
33

34 For field grown plants, control must be maintained over the growing process from  
35 planting through harvesting and over the disposition of remaining crops and/or crop residue and,  
36 if required, over the subsequent use of the field if for growth of food or feed or as a pasture  
37 during subsequent seasons. Control measures should include an accounting of seed that is  
38 transferred from seed bank storage to the field for planting, or for archiving. Records should be  
39 maintained on plant growth rates, environmental conditions (e.g. daily mean temperatures,  
40 rainfall, sunlight hours), and the presence of weeds, insects or animals from the time of planting  
41 to harvest. Conditions for determining when the plants are to be harvested should be clarified  
42 prior to planting.  
43

44 Documentation of the size and location of all sites where the bioengineered plants will be  
45 grown, of the control of pollen spread, and of the subsequent use of the field and destruction of  
46 volunteer plants in subsequent growing seasons should be maintained and provided to the NRA.

1 Such documentation may also be required and/or requested by other national regulatory agencies  
2 such as those for the environment, wildlife, or agriculture.

3  
4 Appropriate confinement procedures should be in place for transport of the source  
5 material from the field or greenhouse to the production facility. During transport, containers of  
6 harvested material should carry a label that clearly indicates that the material is not to be used for  
7 food or feed purposes.

8  
9 In-process wastes, rejected in-process material, and residual source plant material from  
10 the purification process should be treated to inactivate the regulated product prior to its disposal.  
11 The waste should be disposed of in a manner to ensure that the material will not enter the human  
12 or animal food chain and in concordance with regional practices.

13  
14 The in-process monitoring of sterility or for mycoplasma contamination would be  
15 inappropriate for any green plant material prior to appropriate purification and filtration steps.  
16 However, appropriate measures should be in place to minimize the bioburden or other extraneous  
17 contamination.

### 18 19 ***A.5.1.3 Production from ascites***

20  
21 The production of mAbs in animal ascites for use in humans is strongly discouraged; however, it  
22 is recognized that this method may be required under unique circumstances and strong scientific  
23 justification should be provided for not using in vitro cell culture or plant-based methods. When  
24 the ascites method of production is used, 3Rs principles of animal welfare (Reduction,  
25 Refinement, Replacement) effort must be considered. Discomfort, distress, and pain must be  
26 avoided as much possible, and any animals under distress should be euthanized.

27  
28 Animals should be weighed prior to injection of hybridomas and their weight gain  
29 monitored daily. If substances other than pristane are used to prime the animals to facilitate the  
30 growth of hybridomas, the NRA should approve them. Harvesting of the ascites fluid should be  
31 done under anesthetic and before the abdominal distension becomes distressful to the animal, or  
32 its normal activity, respiration, and food or water intake are negatively impacted. A maximum of  
33 4 harvests (taps) may be drawn from the same animal prior to its euthanization. If the collected  
34 exudate is bloody or cloudy it must be discarded and the animal humanely euthanized  
35 immediately.

### 36 37 **A.5.2 Conjugation**

38  
39 Several multi-step chemical and enzymatic methods for the conjugation of mAbs to small  
40 molecule drugs (55-58) or PEG (59-61) have been described. The choice of conjugation process  
41 should be justified and consider the purpose and function of the final product. The linker selected  
42 should remain stable during circulation so as not to inadvertently release the conjugated payload  
43 prior to reaching the target of interest.

44  
45 Methods that conjugate in random positions of the mAb are less desirable due to the  
46 generation of broadly heterogeneous conjugate mixtures with variable lot-to-lot consistency,

1 pharmacological effects, potency, efficacy and stability. Methods employing more specific  
2 conjugation chemistry are better able to control the site of modification, the ratio of the payload  
3 to mAb, and have better batch consistency. The method selected for conjugation should be  
4 approved by the NRA.

5  
6 All individual components used in the conjugation process must be controlled for  
7 identity, purity, and stability. Potency of the mAb, as well as the potency or unit of biological  
8 activity of a pharmacologically active payload, should also be confirmed prior to their  
9 conjugation. PEG molecular size distribution, monofunctionality, linearity or branch size should  
10 be verified. Characteristics that contribute to the safety and efficacy of the mAb conjugate that  
11 are important in the control for its release and stability should be determined during the  
12 development process. These characteristics can include the payload:mAb ratio, potential  
13 conjugation sites, unintended or incomplete conjugations, impact of conjugation on mAb  
14 recognition of the antigen binding site and its affinity, functionality of the Fc region, and changes  
15 to size or charge variants. Due to the increased complexity of conjugated mAbs, multiple assays  
16 are likely to be needed to ensure that all aspects of its mechanism of action are properly  
17 controlled.

18  
19 Both the conjugation method and the control procedures should be well established to  
20 ensure the reproducibility of the reaction and the production of stable and safe mAb conjugates  
21 prior to their clinical evaluation. The conjugation process should be monitored and analysed for  
22 any unique reaction by-products such as residual unreacted functional groups or their derivatives  
23 that are potentially capable of reacting in vivo and may be present following the conjugation  
24 process. The manufacturing process should be validated and the limits for by-products and  
25 unreacted activated functional groups remaining at the end of the conjugation process and  
26 purification process should be agreed with the NRA. For radio-labelled conjugates, the  
27 development and validation of the conjugation process may be done using equivalent non-  
28 radioactive labels.

### 29 30 **A.5.3 Purification**

31  
32 All purification processes should be evaluated to understand their capacities to sufficiently  
33 reduce or remove impurities (both product and process-related) and contaminants, as well as the  
34 potential impact of the process on mAb quality and aggregation. Purification of mAbs is usually  
35 done over multiple steps using a combination of methods that may include centrifugation,  
36 filtration, ultrafiltration, affinity chromatography (e.g. protein A or protein G), ion exchange  
37 chromatography, or other liquid chromatographic methods. The purification process must also be  
38 assessed for its potential to introduce impurities (e.g. column leachates or components of the  
39 running buffer).

40  
41 Conditions for each purification step should be clearly defined. Some aspects that should  
42 be considered include:

- 43
- 44 • pressure limits for filtration and chromatography steps;
- 45 • column and resin load capacities for substance and impurities;
- 46 • resin or filter lifetime;

- 1 • height equivalent to theoretical plate;
- 2 • potential impurity carry-over and introduction of contaminants;
- 3 • substance purity and yield following each step;
- 4 • leaching of protein A or protein G from chromatography columns;
- 5 • endotoxin and bioburden;
- 6 • chromatographic profile.

7  
8 In order to help speed the development of products to patients during emerging outbreaks  
9 the use of existing modular data for purification process validation (e.g., impurity clearance)  
10 from other mAb products manufactured using the same processes can be considered. However,  
11 this should be discussed with the NRA as early as possible during development.

12  
13 Following purification, the bulk mAb substance can be held under appropriate  
14 environmental conditions until further processing. Selected hold times and conditions should be  
15 supported by data and consider all stability indicating attributes. These should be approved by  
16 the NRA. Refer to section A.12 for further discussion on the hold time and storage of mAb  
17 substances.

#### 18 19 *A.5.3.1 Validation of procedures for removing host cell proteins (HCPs)*

20  
21 HCPs will likely comprise the largest percent and most physicochemically diverse range of the  
22 impurities that must be removed in the production of the drug substance and their diversity will  
23 vary between cell types, growth conditions, whether the mAb is secreted or derived from lysed  
24 cells, and any pre-purification processing steps. Their removal is crucial in order to avoid the  
25 potential for inducing an undesired immunological response, preventing an adjuvant effect they  
26 may confer on the mAb substance, as well as preventing their potential impact on the mAb  
27 substance quality (for example, degradation by enzymatically active HCPs).

28  
29 Spiking studies can be used in the validation of the purification process for the removal  
30 of HCPs; however, these studies should be considered carefully. As the types of HCPs present  
31 differ over the range of the culture/growth period and are influenced by environmental  
32 conditions, it is important that the validation studies use materials (a model HCP solution) which  
33 are derived from production processes that are as closely representative of the intended  
34 production process of the mAb substance. As purification processes for mAbs involves several  
35 sequential steps, it may not be appropriate, or useful, to evaluate each purification step using the  
36 same model HCP solution. More appropriate procedures could include studies in which a limited  
37 volume of the model HCP solution has been diluted with an eluate from a prior purification step,  
38 and/or a study in which each purification step is evaluated when the prior step is used under best-  
39 case and worst-case conditions (62).

40  
41 Implementation of sensitive detection and quantification methods is crucial for the  
42 successful validation of the purification process and understanding of its capacities and  
43 limitations. Some discussion on HCP detection methods is provided in section A.5.5.8.1.  
44 Although commercially available ELISA kits for HCPs may be used for their quantification, they  
45 may not detect a sufficient range of proteins and, therefore, should be carefully assessed for the  
46 capacity. It is recommended that product-specific HCP antiserum be developed and qualified for

1 use in an HCP ELISA prior to seeking marketing authorization; however, commercially  
2 available kits may be useful during early manufacturing development stages.

#### 3 4 ***A.5.3.1 Validation of procedures for removing residual cellular DNA***

5  
6 Acceptable limits on the amount of residual cellular DNA (rcDNA), as well as points to be  
7 considered concerning the size of rcDNA in a rDNA-derived biotherapeutic, are discussed in  
8 section 5.2.2 of the WHO *Recommendations for the evaluation of animal cell substrates for the*  
9 *manufacture of biological medicinal products and for the characterization of cell banks* (52).  
10 These recommendations indicate an acceptable upper limit of rcDNA of 10 ng per parenteral  
11 dose; however, it is important to take into consideration additional factors such as DNA fragment  
12 size as well as any inactivating steps that may be included in the manufacturing process.  
13 Acceptable daily and/or treatment cycle limits for rcDNA should be discussed with the NRA.

14  
15 Validation studies for the removal of rcDNA should be performed by spiking source  
16 materials with known amounts of representative DNA of sufficiently large quantity to monitor  
17 the limits of the removal capacity. At each purification step, the product should be tested for the  
18 content of the added DNA. On the basis of the overall reduction obtained in DNA content (the  
19 “reduction factor”), a calculation should be made to estimate the highest expected amount of  
20 rcDNA per single human dose (or diagnostic intervention) in the final product and, in the case of  
21 products which require multiple administrations, of the highest expected amount of DNA per full  
22 treatment cycle.

#### 23 24 ***A.5.3.2 Validation of procedures for removing viruses***

25  
26 Validation studies must be conducted on the ability of the manufacturing process to remove  
27 and/or inactivate viruses and viral particles. Crude material produced before any purification and  
28 subsequent fractions obtained during the various purification steps should be spiked with  
29 appropriate amounts of relevant and/or model viruses.

30  
31 Relevant viruses for validation studies are those that are known, or likely, to contaminate  
32 the source material or products used in the production process. The purification and/or  
33 inactivation process should be shown to remove or inactivate such viruses or viruses of a similar  
34 class. Cell lines derived from rodents usually contain endogenous retroviral particles, which may  
35 be infectious (C particles) or non-infectious (intracisternal A particles). Therefore, it is necessary  
36 to validate the capacity of the purification process to remove murine retroviruses from mAb  
37 preparations obtained from such cells. In such cases, a murine leukemia virus may be appropriate  
38 to use during validation. When human cell lines secreting mAbs have been obtained by  
39 immortalization of B lymphocytes by EBV, it is necessary to check the ability of the purification  
40 process to remove EBV by studies with a suitable herpesvirus.

41  
42 There may be cases where relevant viruses do not have a wide range of physicochemical  
43 properties, or where spiking with relevant viruses is too hazardous. In such cases, validation  
44 studies should be performed with model viruses, although the presence of such viruses in cell  
45 cultures used for mAb production may be unlikely. Preference should be given to viruses that  
46 display significant resistance to physical and/or chemical agents. Reduction factors obtained for



1 such model viruses provide useful information on the ability of the production process to remove  
2 and/or inactivate viruses in general. Suitable model viruses capable of resisting a range of  
3 physicochemical agents include:

- 4
- 5 • small, non-enveloped viruses, such as SV40 (Polyomavirus maccacae 1) or human
- 6 poliovirus 1 (Sabin);
- 7 • medium-to-large enveloped RNA viruses, such as parainfluenza or influenza virus, or a
- 8 murine retrovirus;
- 9 • medium-to-large DNA virus, such as a herpesvirus (e.g. human (alpha) herpesvirus 1 or a
- 10 pseudorabies virus).

11

12 A generic clearance study may be considered where virus removal and/or inactivation is  
13 demonstrated over several steps in the purification process of a model mAb. These data may then  
14 be extrapolated to other mAbs manufactured within the same facility and which follow the same  
15 purification processes and virus removal/inactivation schemes as the model mAb.

16

17 In order to speed products to patients during emerging public health outbreaks the use of  
18 modular data already obtained for viral clearance validation can be considered and discussed  
19 with the NRA. A modular clearance study is one that demonstrates the capacity of each  
20 individual step of the purification process to remove and/or inactivate viruses. This should not  
21 only include assessment of the filtration and chromatography steps, but also the impact of any  
22 pasteurization step, solvents, detergents, or changes to pH which may be used within the  
23 production process. Each module in the purification scheme may be studied independently of the  
24 other modules. If necessary, different model mAbs may be used to demonstrate viral clearance in  
25 different modules. If the purification process of the drug substance differs at any of the virus  
26 removal or inactivation modules from the model mAb, this module must be studied  
27 independently from the model. The other, identical modules in the procedure may be  
28 extrapolated to the drug substance.

29

30 If a viral genome sequence has been found in the MCB, the product may be used on  
31 condition that the purification process is validated for its capacity to reduce the content of the  
32 viral subgenomic fragments in the final product to a level undetectable by hybridization, PCR, or  
33 other genomic amplification methods.

#### 34

#### 35 *A.5.3.3 Validation of procedures for removing impurities*

#### 36

37 The purification process must be demonstrated through specific validation studies to be able to  
38 remove, or sufficiently reduce to an acceptable level, any and all impurities from the drug  
39 substance. The types of the impurities that should be considered are largely dependent on the  
40 production processes and include, but are not limited to:

- 41
- 42 • Any additives which may be used in the culture media or bioreactors (e.g. sera, serum
- 43 substitutes, antibiotics, insulin, IPTG, DMSO, antifoam agent);
- 44 • enzymes which may be used for digestion purposes;
- 45 • agents used in the purification process and columns (e.g., Protein A, solvents used in
- 46 running or elution buffers);

- 1 • reagents used in conjugation reactions, including unconjugated linker, drug and/or PEG
- 2 components;
- 3 • undesired heterogenic mAbs, their aggregates and fragments.

4  
5 For mAbs produced in transgenic plants, if the host species is known to generate  
6 toxicants (e.g., protease inhibitors, hemolytic agents, neurotoxins) then analytical testing, animal  
7 testing, or validation of removal may be required to establish that any residual toxicant levels are  
8 within a safe range in the final product. Where pesticides, fertilizers and/or herbicides may have  
9 been used on the plants or production fields, validation of their removal during the purification  
10 process may be an acceptable alternative to final product safety tests. This should be discussed  
11 with the NRA. Plants may also produce proteases or other enzymes that may cause degradation  
12 to the drug substance and/or impact long-term product stability so care should be taken to  
13 eliminate these as early in the purification process as possible.

#### 14 15 **A.5.4 Intermediates**

16  
17 If the mAb is intended to be modified after purification, such as a conjugation, it is considered to  
18 be an intermediate prior to such modifications. In general, the intermediate should be controlled  
19 as per the purified bulk mAb; however, some testing may be reduced or delayed until after  
20 conjugation or other modification.

#### 21 22 **A.5.5 Control of mAb or mAb conjugate drug substance (purified bulk)**

23  
24 Extensive characterization studies should be conducted on the mAb or mAb conjugate substance  
25 during the development process with the aim of identifying critical quality attributes. Similarly,  
26 process development studies should be conducted to identify individual steps that may impact  
27 the substance and product quality and stability. At a minimum, characterization of the purified  
28 bulk should include physicochemical analysis, biological activity, immunochemical properties,  
29 purity, impurities, contaminants, and quantification. A detailed discussion on characterization is  
30 provided in Appendix 2 of the *WHO Guidelines on the quality, safety and efficacy of*  
31 *biotherapeutic protein products prepared by recombinant DNA technology* (2). For mAb  
32 conjugates it is important to also understand the chemistry of the conjugation process, its control  
33 in achieving a consistent conjugated substance, the impact on the functions of the mAb as well as  
34 on the function of the payload.

35  
36 The selection of appropriate testing requirements and specifications for the control of  
37 purified bulk mAb and mAb conjugate substances should be determined during the  
38 characterization process and be reflective of identified critical quality attributes, production and  
39 purification processes, as well as any chemical or enzymatic modifications and conjugation  
40 reactions. Further discussion on specifications is provided in Appendix 3 of the *WHO Guidelines*  
41 *on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant*  
42 *DNA technology* (2). All methods intended for quality control purposes must be demonstrated as  
43 suitable for their intended purposes during the development process and be validated prior to  
44 application for marketing authorization. The test methods used and specifications for the mAb or  
45 mAb conjugate substance should be discussed with the NRA. At a minimum, purified bulk mAb  
46 and mAb conjugate substances should be evaluated for the following attributes.

1

2 ***A.5.5.1 Appearance***

3

4 The appearance of the purified bulk mAb or mAb conjugate should be examined by a suitable  
5 method and should meet the established specifications for its form and colour. For a dried or  
6 lyophilized preparation, the appearance should also be observed after reconstitution with the  
7 appropriate diluent and should meet the established specifications.

8

9 ***A.5.5.2 Identity***

10

11 The identity tests selected should be specific and based on the mAb or mAb conjugate's  
12 immunological specificity, molecular structure, isotype, light chain composition, other specific  
13 properties and/or presence of any conjugated payload. More than one identity test may be  
14 necessary. Tests selected for identity should possess sufficient specificity to discriminate the  
15 mAb from other products that may be manufactured in the same facility and distinguish between  
16 conjugated and non-conjugated mAbs. Examples of possible methods include target antigen  
17 binding assays, anti-idiotypic immunoassay, Western blot, peptide mapping or analysis using  
18 mass spectrophotometry.

19

20 ***A.5.5.3 pH***

21

22 The pH of each batch should be tested. The results should be within the established range based  
23 on the formulation pH target as supported by formulation development data. The pH must also  
24 be compatible with stability data.

25

26 ***A.5.5.4 Protein concentration***

27

28 Total protein concentration should be measured using a validated method of suitable sensitivity  
29 and specificity such as by determining the absorbance at 280 nm. Chemical methods, such as the  
30 bicinchoninic acid (BCA), Lowry, or Bradford assays may also be used if acceptable sensitivity  
31 is demonstrated.

32

33 ***A.5.5.5 Potency***

34

35 Potency assays should provide a quantitative measure of a mAb or mAb conjugate's activity, or  
36 activities, which are relevant to its mechanism of action. The use of assays that reflect its  
37 mechanism of action in the clinical situation is preferable but is not always possible, or  
38 necessary, when the assays are intended for quality control and release testing purposes. Multiple  
39 potency assays may be required to assess all of its relevant functions. This could include, for  
40 example, assays for binding to the target antigen as well as evaluating Fc function. For bispecific  
41 or multi-specific mAbs, the dual or multiple binding capacity to each of the target antigens  
42 would need to be confirmed.

43

44 Potency assays should be sufficiently sensitive so as to detect differences in the mAb or  
45 mAb conjugate of potential clinical importance. Potency assays are also an important measure of  
46 manufacturing consistency and should be sensitive enough to detect changes in the mAb or mAb

1 conjugate that may impact its activity and function(s), such as binding capacity or ADCC. The  
2 more direct assays for assigning potency to mAbs or mAb conjugates are usually in an ELISA  
3 format to assess the binding capacity to its relevant antigen(s). Potency assays may be more  
4 technically complex methods involving, for example, SPR or flow cytometry, but may also be  
5 cell-based assays such as those using a reporter cell line or measure viral neutralization. Potency  
6 assays that include effector functions, such as ADCC or CDC mechanisms of action, should also  
7 be considered where a mAb activity is dependent on more than antigen recognition and binding.  
8 The selection of the appropriate assay(s) used for monitoring the potency of mAbs should be  
9 discussed with the NRA.

10  
11 For cell-based assays that utilize a continuous cell line, a cell bank system should be  
12 generated and qualified. Any reporter gene function should also be shown to be stable through  
13 storage and growth of the cells. For virus neutralization assays, a master cell bank of virus  
14 producer cells should be appropriately qualified and used to generate a working cell bank.

15  
16 For mAb conjugates in which the payload has specific pharmaceutical properties (e.g.  
17 antibody-drug conjugates, or radiolabeled conjugates) the assignment of potency should consider  
18 the binding and any effector function of the mAb following conjugation, the pharmacological  
19 activity of payload, as well as the payload to mAb ratio. For some mAb conjugates, the use of a  
20 cell-based assay for assigning potency may be sufficient, such as when the mechanism of action  
21 reflects both mAb binding and payload functions.

22  
23 Potency assays for mAbs are usually expressed as a percent of activity relative to  
24 established and qualified reference standards which themselves are linked to product batches  
25 used during preclinical and clinical trials. Specifications should consider historical release and  
26 stability batch data, clinical experience, manufacturing history and capability, as well as the  
27 analytical capability of the methods used. In general, the expected acceptance criteria range for  
28 assays which assess ligand binding (e.g. ELISA or SPR) is within 80% - 120% of the reference  
29 standard whereas for functional in vitro assays (e.g. cell based reporter assays, or assays which  
30 monitor ADCC or CDC) the results should be within 70% - 130% of the reference. Acceptance  
31 criteria outside of these ranges should be justified and would be based on the assay type, assay  
32 variability, and historical batch data of the mAb.

33  
34 Although in vivo methods can be used to determine potency, these assays are mostly  
35 done during the product development phase and may be inadequate for the purposes of quality  
36 control and release testing. Animal-based potency assays tend to have a much higher variability  
37 and may lack the sensitivity necessary for assuring the consistent quality of a mAb. It is  
38 important to ensure that, if in vivo methods are used, the mAb target(s) are expressed within the  
39 animal and that any inter-species differences between the animal and human biology are  
40 considered. The selection of animal methods for release testing purposes must adhere to the  
41 principles of 3Rs (Reduction, Refinement and Replacement), and should also be discussed with  
42 the NRA.

#### 43 44 *A.5.5.6 Heterogeneity profile (purity)*

45

1 Following purification, purified bulk mAbs and mAb conjugates will comprise heterogenous  
2 populations of molecular species with variants in mass, charge, glycosylation, and other  
3 parameters (25, 63-65). The types of variants encountered can be influenced by a number of  
4 factors such as the cell or plant substrate, culture media and environmental conditions during  
5 growth, the purification processes, as well as by any additional chemical or enzymatic  
6 modifications. Collectively, the variants provide a heterogeneity profile, or “fingerprint”, which  
7 is unique to each substance and manufacturing process, and on which the substance  
8 specifications are established.

9  
10 At a minimum, heterogeneity profiles should be assessed for mass, charge, and  
11 glycosylation variants for each batch of purified bulk mAb. An evaluation on the distribution of  
12 conjugate variants should also be conducted for purified bulk mAb conjugates. Although a  
13 number of techniques may be employed to measure the heterogeneity profile, the more  
14 commonly used quantitative tools include various HPLC techniques (such as anion exchange,  
15 cation exchange, size exclusion, and reversed-phase chromatography), capillary electrophoresis  
16 (CE), and isoelectric focusing electrophoresis (65-68). Nuclear magnetic resonance, mass  
17 spectrometry and circular dichroism may provide additional characteristics and information  
18 regarding the types of heterogeneous mAbs. Heterogeneity profiles must be assessed using a  
19 variety of analytical methods with acceptable limits based on outcomes from characterization  
20 studies and in comparison to preparations used in establishing manufacturing consistency. The  
21 selection for appropriate methods for evaluating the heterogeneity and their acceptance criteria  
22 should be discussed with the NRA.

23  
24 If the purified bulk mAb or mAb conjugate is well characterized and the manufacturing  
25 process has been demonstrated to be well controlled, then it may be feasible to reduce the  
26 number of tests required for purity and heterogeneity assessment. However, any subsequent  
27 changes to the materials used, equipment, manufacturing process, purification method, and/or  
28 conjugation chemistry may warrant the re-establishment of appropriate tests.

#### 30 ***A.5.5.7 Substance-related impurities***

31  
32 Substance-related impurities should be monitored with each batch of purified bulk mAb or mAb  
33 conjugate. These impurities could include antibody fragments, aggregates, charge variants  
34 (deamidation, glycation, glucuronylation, sialylation, intact C-terminal lysine and unconverted  
35 N-terminal glutamine), oxidized species, and free glycans. For purified bulk mAb conjugates,  
36 any unconjugated mAb, free payload, as well as free linker-payload conjugate would also be  
37 considered as product-related impurities. Appropriate specifications should be established and  
38 based on knowledge gained during characterization studies, stability batch data, clinical  
39 experience, manufacturing history and capability, analytical method capability, regulatory  
40 expectations, safety, and any compendial requirements for protein-based products.

41  
42 The methods employed for assessing the heterogeneity profile (section A.5.5.6) may also  
43 be valuable in monitoring for substance-related impurities. MAb aggregate formation can also be  
44 monitored using techniques such as dynamic light scattering, optical density at visible  
45 wavelengths, nephelometry, light obscuration, or flow imaging (69, 70).

### 1 *A.5.5.8 Process-related impurities*

2  
3 Selection of appropriate tests for the detection of process-related impurities should consider all  
4 manufacturing steps commencing from the WCB/WSB. Acceptable limits for such impurities  
5 should be justified by the minimum levels achievable by the purification process, as well as on  
6 their concentration after dilution of the purified bulk into the final product, the volume of  
7 administration, and whether the product is intended for single or repeated administration.  
8 Specifications for the impurities should be discussed with the NRA with acceptable limits based  
9 on the risk assessment.

10

#### 11 *A.5.5.8.1 Host cell proteins*

12

13 HCPs are most commonly measured using an ELISA platform with polyclonal anti-HCP  
14 antiserum. As the performance of the assay is limited by the quality and specificity of the anti-  
15 serum, the assay will not accurately reflect the true level of HCPs if the antiserum does not  
16 recognize the majority of the HCPs or if the signal is dominated by antibodies to only a few  
17 proteins present in the sample being tested. Although commercial assay kits are available for the  
18 detection of HCPs from some cells (e.g. CHO cells and E. coli), these assays may not detect the  
19 HCPs unique to the cells grown under a manufacturer's bioreactor environmental conditions or  
20 to be sufficiently specific to the HCPs that may co-elute with the mAb during purification. The  
21 development of process-specific HCP antiserum raised against a harvest and/or from early in the  
22 purification process and from the same cell line as used in the manufacture of the mAbs but  
23 transfected with an empty vector are recommended.

24

25 The characterization and understanding of the estimated percent coverage of HCPs which  
26 the antiserum/ELISA are detecting is important and should be provided for marketing  
27 authorization. The use of 2-dimensional gel electrophoresis methods is useful in this exercise  
28 but, alone, does not sufficient information. Mass spectrophotometry analysis of HCPs is  
29 recommended as an orthogonal approach in order to identify individual proteins, quantify the  
30 more abundant ones, and thereby support the risk assessments.

31

32 Although there is no clearly established safe or acceptable level of HCPs, achieving  
33 levels below 100 ppm (< 100 ng/mg mAb protein) are generally recognized as sufficient.  
34 However, the acceptable level for any mAb product must be based on a risk assessment and will  
35 also depend on dose and frequency of administration.

36

#### 37 *A.5.5.8.2 Other process-related impurities*

38

39 Other potential process-related impurities to consider from the cell culture include rcDNA,  
40 cellular metabolites and cell culture media components. A nucleic acid amplification technique,  
41 such as qPCR, or some colorimetric methods may be suitable to detect and quantify the level of  
42 any rcDNA in the purified bulk mAb or mAb conjugate. Testing for beta-glucans should also be  
43 considered, particularly if the host cell is known for generating the oligosaccharide or if cellulose  
44 filters are used downstream (71).

45

1 Validated quantitative methods must be in place to test for trace levels of any antibiotic  
2 that is used in the culture media or at any other step in the manufacturing process. Similarly,  
3 methods should be established for the detection and quantification of cell culture components  
4 such as inducers, enhancing agents, surfactants, antifoam reagents, chelators, or solvents. As the  
5 purified bulk substance can be a difficult matrix for some detection methods, the sensitivity of  
6 the technique should be demonstrated with spike and recover studies.

7  
8 Downstream processing steps, such as purification and conjugation, are likely to be  
9 important sources of impurities and may include enzymes, chemical or biochemical processing  
10 reagents, buffer components, stabilizers, leachates, elemental impurities, chromatography media  
11 (such as organic solvents or DMSO), and ligands which may leach from affinity columns (e.g.  
12 Protein A or Protein G). For conjugated mAbs, methods must be in place to detect residual  
13 unbound payload as well as the linker arm and all reagents used in the conjugating reaction.

14  
15 For plant-derived mAbs, methods should be established and validated for the detection of  
16 any fertilizers, herbicides and pesticides which may have been applied to the field or to the crop  
17 before or during the growth of transgenic plants used in the production process. The potential for  
18 plants to produce agents which may pose a safety risk to humans or impact the product quality or  
19 stability should also be assessed and identified during the development programme. These plant-  
20 derived agents can include proteolytic enzymes, lectins, polysaccharides, and/or secondary  
21 metabolites.

#### 22 23 ***A.5.5.9 Sterility or bioburden testing***

24  
25 The purified bulk mAb or mAb conjugate should be tested for bacterial and fungal bioburden or  
26 sterility according to the methods described in Part A, section 5.2 of the WHO *General*  
27 *requirements for the sterility of biological substances* (54), or using methods approved by the  
28 NRA. Any purified bulk substance that is contaminated should be discarded and not subject to  
29 re-purification or filtration. If a preservative or other agent has been added to the purified bulk  
30 then appropriate measures should be taken to ensure it does not interfere with the tests.

#### 31 32 ***A.5.5.10 Endotoxin***

33  
34 The endotoxin content of each lot of purified bulk mAb or mAb conjugate should be determined  
35 and shown to be within limits agreed with the NRA. Suitable in vitro methods include the  
36 Limulus amoebocyte lysate (LAL) test or monocyte activation test (MAT). For purified bulk  
37 substance that may have non-endotoxin pro-inflammatory impurities, such as peptidoglycan or  
38 beta-glucan, the use of a MAT or other method should be considered. The method development  
39 or validation studies must include an evaluation of low endotoxin recovery from spiked samples.

#### 40 41 ***A.5.5.11 Ratio of payload to mAb (if applicable)***

42  
43 For purified bulk mAb conjugates, the ratio of the payload to mAb (expressed, for example, as  
44 g/g or mol/mol) in the purified bulk conjugate should be calculated. For this ratio to be a suitable  
45 marker of conjugation, the content of each of the conjugate components prior to their use should  
46 be known. For each purified bulk mAb conjugate, the ratio should be within the range approved

1 by the NRA for that particular conjugate and should be consistent with the ratio in the product  
2 that has been shown to be effective in clinical trials.

### 4 **A.5.6 Preparation and control of the final bulk**

6 Excipients selected and their concentrations used in the final bulk should be shown to have no  
7 deleterious effects on the mAb or mAb conjugate function, structure, or stability, and should not  
8 promote their aggregation. The excipients should cause no unexpected adverse reactions in  
9 humans or induce adverse reactions to the mAb or mAb conjugates. The types and  
10 concentrations of all excipients should be approved by the NRA.

#### 12 ***A.5.6.1 Preparation***

14 The final bulk is prepared by mixing a suitable quantity of the purified bulk mAb or mAb  
15 conjugate with all other product constituents, which may include a stabilizer, bulking agent,  
16 preservative and/or other purified bulk mAbs or mAb conjugates. The inclusion of excipients is  
17 optional; however, if included, their effect on mAb immunogenicity and potential for causing  
18 aggregates should be assessed during the product development process and the final  
19 concentration must be considered safe for the administration to humans.

21 The final bulk should be prepared using a validated process and should meet the  
22 specifications based on the quality attributes of product lots that have been shown to be safe and  
23 efficacious in clinical trials. The maximum hold time and storage conditions of the final bulk  
24 prior to filling must be clearly defined.

#### 26 ***A.5.6.2 Test for ratio of combined mAbs (if applicable)***

28 If two or more mAbs and/or mAb conjugates are combined during the preparation of the final  
29 bulk in the manufacture of a mAb cocktail product, a test must be in place to ensure the proper  
30 ratio of each mAb and/or mAb conjugate substance.

#### 32 ***A.5.6.3 Test for bacterial and fungal sterility***

34 The final bulk should be tested for bacterial and fungal sterility according to the methods  
35 described in Part A, sections 5.1 and 5.2 of the WHO *General requirements for the sterility of*  
36 *biological substances* (53), or using methods approved by the NRA. If a preservative has been  
37 added to the final bulk, appropriate measures should be taken to prevent it from interfering with  
38 the tests.

## 41 **A.6 Filling and containers**

43 The relevant guidance provided in WHO *good manufacturing practices for pharmaceutical*  
44 *products: main principles* (41) and WHO *good manufacturing practices for biological products*  
45 (42) should be followed.



1 All containers and container closure systems used for the substance and product must be  
2 tested for compatibility with the drug substance(s) and final product formulation, and must also  
3 be in compliance with NRA requirements, such as for biological reactivity, leachables, and  
4 extractables. Assurance for the absence of TSE (50) should be provided if any animal-derived  
5 materials (e.g. colourants made from tallow, or fatty acids used in polymer production) are used  
6 in the manufacture of the container or closure. Integrity testing of the containers and closures  
7 must be done to ensure they can maintain the stability and sterility of the contents for the  
8 duration of the product shelf-life.

9  
10 Care should be taken to ensure that the materials of which the containers and closures are  
11 made (and, if applicable, the transference devices) do not adversely affect the quality of the mAb  
12 product. In particular, as mAbs in high concentration have a propensity for forming aggregates,  
13 the containers and closures should not induce or otherwise promote aggregation. To this end, a  
14 container closure integrity test and assessment of extractables and/or leachables for the final  
15 container closure system are generally required for the qualification of containers, and may be  
16 needed as part of stability assessments.

17  
18 If multi-dose vials are used and the mAb products do not contain a preservative then their  
19 use should be time-restricted following the first withdrawal. In addition, the multi-dose container  
20 should prevent microbial contamination of the contents after opening. The manufacturers should  
21 provide the NRA with adequate data to prove the stability of the product under appropriate  
22 conditions of storage and shipping.

## 23 24 25 **A.7 Control of the final product**

### 26 27 **A.7.1 Inspection of the final containers**

28  
29 All filled final containers should be inspected as part of the routine manufacturing process.  
30 Those containers showing abnormalities (e.g. vial defects, improper sealing, or the presence of  
31 endogenous or exogenous particles) should be discarded. The test should be performed against  
32 both black and white backgrounds, and according to pharmacopoeial specifications.

### 33 34 **A.7.2 Control tests on the final lot**

35  
36 The following tests should be performed on each final lot (from the final container) of mAb,  
37 mAb conjugate, or mAb cocktail and the tests used should be validated and approved by the  
38 NRA. The method development and validation process should include a demonstration that any  
39 excipients (e.g. preservatives or stabilizers) included in the final formulation do not interfere  
40 with the assays. The permissible limits for tests listed under this section should be justified and  
41 approved by the NRA, and assay results should support the label claim. Once consistency of  
42 production is demonstrated, it may be possible to omit some tests if sufficient justification is  
43 provided and pending agreement with the NRA.

#### 44 45 ***A.7.2.1 Appearance***

1 The appearance of the final container and its contents should be verified using a suitable method  
2 and should meet the established criteria with respect to form and colour. For lyophilized or  
3 freeze-dried products, their appearance should be verified before and after reconstitution, and  
4 should meet the established criteria.

#### 5 6 ***A.7.2.2 Identity*** 7

8 Identity tests on the mAb, mAb conjugate, or mAb cocktail should be performed on each final  
9 lot. Their immunological specificity must be verified. Testing should include the mAb capacity  
10 to react with the target antigen, the isotype, and light chain composition. Additional testing could  
11 include Western blot, glycosylation analysis, reporter-cell assay, and/or mass spectrophotometric  
12 methods. For mAb cocktails, release testing methods should include an identity method that  
13 demonstrates the presence of each individual antibody and a quantitative method to confirm their  
14 ratio.

#### 15 16 ***A.7.2.3 pH and osmolality*** 17

18 If the product is a liquid preparation, the pH of each final lot should be tested, and the results  
19 should be within the range of values approved by the NRA. For a lyophilized preparation, the pH  
20 should be measured after reconstitution using the same diluent recommended during clinical use.

21  
22 The osmolality of the final lots should be determined and shown to be within the range  
23 considered to be safe for parenteral administration to humans and agreed with the NRA. The test  
24 for osmolality may be omitted once consistency of production is demonstrated or justification is  
25 provided, with the agreement of the NRA.

#### 26 27 ***A.7.2.4 Moisture content (if applicable)*** 28

29 If the final product is a lyophilized preparation, the level of residual moisture should be  
30 determined, and the results should be within the limit agreed with the NRA.

#### 31 32 ***A.7.2.5 Protein content*** 33

34 Total protein concentration should be measured using a method of suitable sensitivity and  
35 specificity. Chemical methods, such as the bicinchoninic acid, Lowry, or Bradford assays may be  
36 used, or protein concentration may be determined via absorbance at 280 nm. Protein  
37 concentration of the final product must be within +/- 10% of the labeled claim.

#### 38 39 ***A.7.2.6 Heterogeneity profile (purity)*** 40

41 The heterogeneity profile should be confirmed in the final product as being similar to the drug  
42 substance (section A.5.5.6). Some attributes which should be considered on the final product  
43 consistency include the size distribution, charge heterogeneity, and glycosylation variants.  
44 Conjugated mAbs should also be verified for the heterogeneity of the payload-to-mAb ratio. The  
45 number of methods used to assess the heterogeneity may be reduced if the impact of the

1 formulation and filling processes are clearly characterized and demonstrated to have little effect;  
2 however, this should be appropriately justified and discussed with the NRA.

#### 3 4 **A.7.2.7 Product-related impurities**

5  
6 Product-related impurities, such as aggregates, fragments, and non-consensus glycosylated  
7 species, should be measured in the final product. Some product-related impurities or post  
8 translational modifications may be measured in drug substance only if the drug product  
9 manufacturing process is demonstrated to not have an impact on the impurities and post  
10 translational modifications. HPLC (for mAb and mAb conjugates) and capillary electrophoresis  
11 or SDS-PAGE (for mAb fragments) are common methods of choice for quantitating these  
12 impurities, although other techniques may also be used.

13  
14 As mAbs are susceptible to aggregation, each final lot should be examined for particulate  
15 matter and aggregate content at lot release and at the end of its shelf-life unless it can be shown  
16 that the test is not necessary.

#### 17 18 **A.7.2.8 Process-related impurities**

19  
20 Measurement of process-related impurities, such as impurities from excipients or bulking agents,  
21 the container closure system, or from other potential sources during the preparation of the final  
22 bulk and from vial filling, should be considered. The control of process-related impurities should  
23 be demonstrated if not measured in the final product. If clearance of process-related impurities  
24 has been demonstrated, or the impurity is controlled as an in-process control or tested in the  
25 purified bulk, there may be justification to exclude it as a release test in the final lot.

26  
27 For mAb conjugate-containing products, a limit should be set for an acceptable amount of  
28 unbound (free) payload. An acceptable value should be consistent with the value seen in the  
29 batches used for clinical trials that showed adequate activity and should be approved by the  
30 NRA.

#### 31 32 **A.7.2.9 Excipients**

33  
34 Each final lot should be tested for the presence and concentration of excipients which are critical  
35 to the product stability and sterility, such as any preservative or polysorbate. Testing  
36 requirements for other excipients which may be added to the final product, such as buffer,  
37 surfactant, or bulking agent, should be discussed with the NRA. The test(s) for excipients in the  
38 final lot may be omitted if they were tested in the final bulk prior to filling.

#### 39 40 **A.7.2.10 Sterility**

41  
42 The contents of the final containers should be tested for bacterial and fungal sterility according to  
43 the methods described in Part A, sections 5.1 and 5.2 of the WHO *General requirements for the*  
44 *sterility of biological substances* (53), or using a method approved by the NRA. If the final  
45 product contains a preservative, appropriate measures should be taken to prevent it from  
46 interfering with the tests.

1

2 ***A.7.2.11 Endotoxin or pyrogen content***

3

4 The endotoxin content of the final product should be determined using a suitable in vitro assay  
5 such as a LAL test or MAT. The method selected should demonstrate adequate endotoxin  
6 recovery in the final formulation in order to account for possible interference with the assay.  
7 Although many NRAs expect an endotoxin content less than, or equal to, 5 EU/kg/h in the final  
8 drug product, the potential contribution of endotoxin from any reconstitution buffer or diluent  
9 should also be considered. The endotoxin content should be consistent with levels found to be  
10 acceptable in product lots used during clinical trials and within the limits agreed with the NRA.

11

12 The need for pyrogenicity testing should be determined during the manufacturing  
13 development process and may be required if non-endotoxin pyrogens, such as peptidoglycan and  
14 beta-glucan, are present. The need for pyrogenicity testing should also be evaluated following  
15 any changes in the production process or relevant reported production inconsistencies that could  
16 influence the quality of the product with regard to its pyrogenicity. When required, the MAT or  
17 rabbit pyrogenicity test may be used for monitoring potential pyrogenic activity subject to the  
18 agreement of the NRA.

19

20 ***A.7.2.12 Reconstitution time (if applicable)***

21

22 The reconstitution time should conform to specification if the final product is presented as a  
23 freeze-dried or lyophilized formulation.

24

25 ***A.7.2.13 Extractable volume***

26

27 It should be demonstrated that the nominal volume indicated on the label can consistently be  
28 extracted from the containers whether single-dose or multi-dose.

29

30

31 **A.8 Records**

32

33 The relevant guidance provided in WHO *good manufacturing practices for pharmaceutical*  
34 *products: main principles* (41) and in section 17 of the WHO *good manufacturing practices for*  
35 *biological products* (42) should be followed as appropriate for the level of development of the  
36 product. Written records should be kept of all tests, irrespective of their results. The records  
37 should be of a type from which annual trends can be determined.

38

39

40 **A.9 Retained samples**

41

42 The requirements given in section 16 of WHO *good manufacturing practices for biological*  
43 *products* (42) should apply. A sufficient number of samples from each lot of the product should  
44 be retained for future studies and needs. MAb, mAb conjugate or mAb cocktail lots that are to be  
45 used for clinical trials may serve as reference materials in the future and a sufficient number of  
46 vials should be reserved and stored appropriately for that purpose.

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## A.10 Labelling

The guidance on labelling provided in section 14 of the WHO *good manufacturing practices for biological products* (42) should be followed as appropriate. Labelling should also conform to the national requirements of the region in which the product will be used. All claims on the label must be met by the lot release tests detailed in section A.7.2. As well, the label on the carton, the container and/or the leaflet accompanying each container, should include:

- the name and lot number of the mAb product;
- the volume of one recommended human dose, and the recommended schedule and route(s) of administration;
- the amount of active substance(s) contained in one human dose;
- the number of doses if the product is issued in a multi-dose container, and the storage conditions and shelf-life after opening;
- the name and concentration of any antibiotic and/or other preservative added;
- the temperature recommended during storage and transport;
- the expiry and/or retest date;
- contraindications, warnings and precautions, concomitant product use advice, and potential adverse reactions;
- if applicable, information on the volume and nature of the diluent to be added to reconstitute the lyophilized product; the instruction that any product in a lyophilized form should be used immediately after reconstitution or the approved limited duration of storage of the reconstituted product;

For mAb products containing multiple active substances (e.g. mAb cocktails), the total dose of the product as well as the amount of the individual substances within that dose should be indicated.

## A.11 Distribution and transport

The guidance provided in WHO *good manufacturing practices for pharmaceutical products: main principles* (41) and WHO *good manufacturing practices for biological products* (42) should be followed. Shipping studies from drug substance to drug product manufacturing sites (if at different locations) and release to distribution center should be conducted and validated.

Shipments should be maintained within specified temperature ranges, and packages should contain cold-chain monitors if the temperature needs to be controlled. If it is claimed that a cold-chain is not required then the conditions under which stability has been established (for example, maximum temperature and maximum length of time at that temperature) should be described and data supporting these claims provided. Further guidance on these and related issues is provided in the WHO *Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products* (72).

1

## 2 A.12 Stability testing, storage and expiry date

3

4 **A.12.1 Hold time**

5

6 Hold time studies for any intermediates or process hold steps should be conducted as part of  
7 manufacturing process validation (2) and reflect the holding time at each stage. The impact of  
8 hold times and their environmental conditions should be assessed on the quality of the substance  
9 and/or final product. Hold times should be established and assigned to all in-process materials  
10 during mAb production.

11

12 **A.12.2 Stability testing**

13

14 Stability programs for mAb substances, conjugates, and products should be initiated early in the  
15 development process. Stability study protocols and results supporting the stability claims over  
16 the shelf-life period must be provided to the NRA. Recommended storage conditions for  
17 substances and products should be based on the stability data.

18

19 In order to speed products to patients during emerging public health outbreaks, gathering  
20 stability data while the mAb product is in clinical use may be allowed. Any knowledge gained  
21 with the stability of other mAbs which differ only in their antigen binding domain and which  
22 have been manufactured using the same technology platform may also provide valuable insight  
23 into the stability of novel products intended for emerging public health outbreaks. The NRA  
24 should be consulted regarding this approach.

25

26 Stability-indicating parameters should be defined or selected appropriately according to  
27 the stage of production. When changes are made in the production procedure that may affect the  
28 stability of the product, further stability studies shall be conducted to determine the validity  
29 period of the new product. In such a case, the NRA may agree to the new validity period based  
30 on the results of accelerated-degradation tests.

31

32 For radio-labelled mAbs, stability studies may be conducted using non-radioactive labels  
33 and limited to the expected duration of time in which the isotope label is considered  
34 therapeutically active.

35

36 Accelerated stability and forced-degradation studies are recommended, and may be  
37 required by NRAs for a marketing application. These studies provide additional information on  
38 the overall characteristics of the mAb substance(s) and product and to help identify stability-  
39 indicating methods suitable for on-going stability studies. This information may also be useful in  
40 assessing comparability if, or when, the manufacturer plans to make future changes to the  
41 manufacturing process.

42

43 For mAb product licensure, the stability and expiry date of the product in its final  
44 container, when maintained at the recommended storage temperature, should be demonstrated to  
45 the satisfaction of the NRA using final containers from at least three final lots made from  
46 different mAb bulks. Fewer data are likely to be available during clinical trials; however, the

1 stability of the mAb product under the proposed storage conditions should be demonstrated for at  
2 least the expected duration of the clinical trial.

3

4 Following licensure, ongoing monitoring of the mAb product stability is required to  
5 support shelf-life specifications and to refine the stability profile. Data should be provided to the  
6 NRA according to local regulatory requirements.

7

8 The final stability-testing programme should be approved by the NRA and should include  
9 an agreed set of stability-indicating parameters, procedures for the ongoing collection and  
10 sharing of stability data, and criteria for rejecting mAb product lots. In-use stability and  
11 compatibility (where applicable, for example with infusion sets) should also be specified and  
12 justified with adequate data generated under real-time conditions.

13

#### 14 **A.12.2 Storage conditions**

15

16 Storage conditions should be fully validated. The mAb product should have been shown to  
17 maintain its potency for a period equal to that from the date of release to the expiry date. During  
18 clinical trials, this period should ideally be at least equal to the expected duration of the clinical  
19 trial.

20

#### 21 **A.12.3 Expiry date**

22

23 The expiry date should be based on the shelf-life supported by stability studies and should be  
24 approved by the NRA. The expiry date should be based on the date of manufacturing of the final  
25 bulk, date of filling or the date of the first valid potency test on the final lot.

26

27

### 28 **Part B. Recommendations for NRAs**

29

#### 30 **B.1 General recommendations**

31

32 The guidance for NRAs and national control laboratories (NCLs) given in the WHO *Guidelines*  
33 *for national authorities on quality assurance for biological products* (47) should be followed.

34 These guidelines specify that no new biological product should be released until consistency of  
35 lot manufacturing and quality has been established and demonstrated by the manufacturer. The  
36 detailed production and control procedures, as well as any significant changes in them that may  
37 affect the quality, safety or efficacy of a mAb product, should be discussed with and approved by  
38 the NRA. For control purposes, any relevant international reference preparations currently in  
39 force should be obtained for the purpose of calibrating national, regional and working standards  
40 as appropriate. The NRA and/or NCL may obtain from the manufacturer the product-specific  
41 and/or working references and reagents which may be used for lot release testing purposes.

42

43 Consistency of production is an essential component in the quality assurance of mAb  
44 products. The NRA should monitor production records and quality control test results for clinical  
45 lots, as well as for a series of consecutive lots of the final bulk (i.e. the drug substance) and/or  
46 final product.

1  
2 In addition, the NRA should satisfy itself with the data gathered for ensuring therapeutic  
3 effect and safety in humans and approve:

- 4
- 5 • all methods used in the manufacture of mAb products;
  - 6 • the criteria for establishing manufacturers' reference materials;
  - 7 • all tests for extraneous agents and for total protein;
  - 8 • all tests for preservatives and for agents used for purification or during other stages of  
9 manufacture;
  - 10 • tests used to determine distribution of molecular size;
  - 11 • tests used for determining the potency of the mAb and define the acceptable range of  
12 estimated mean values and the fiducial limits;
  - 13 • the dose to be administered;
  - 14 • the concentration of preservative and excipients in the final product, if added;
  - 15 • the purity of the final product;
  - 16 • the validity period;
  - 17 • the statements concerning storage temperature and expiry date appearing on the label.
- 18

19 The NRA should be satisfied that the results of all tests, including those done for  
20 validating the process of manufacture, are satisfactory and that consistency of production and  
21 testing have been established.

## 22

### 23 **B.2 Release and certification**

24  
25 MAb-containing products shall be released only if they fulfil the requirements of Part A of this  
26 guideline. A statement signed by the appropriate official of the national control authority shall  
27 certify whether the final lot of mAb product in question meets all national requirements as well  
28 as those indicated in Part A. The certificate shall state the lot number (number appearing on the  
29 labels of the containers), the number under which the lot was released and the expiry date.

30  
31 If the product has a very short validity period (e.g. radiolabeled antibody), a national  
32 certificate may not be required for the release of each final product.

### 33

### 34

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