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6 **Guideline on Immunogenicity assessment of**
7 **biotechnology-derived therapeutic proteins**
8 **Draft**

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10 This guideline replaces 'Guideline on Immunogenicity assessment of biotechnology-derived therapeutic
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12 Comments should be provided using this [template](#). The completed comments form should be sent to
13 BMWP.secretariat@ema.europa.eu

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53 **Executive summary**

54 The number of biological/biotechnology-derived proteins used as therapeutic agents is steadily
55 increasing. These products may induce unwanted immune responses, which can be influenced by
56 various factors, including patient- and disease-related factors as well as product-related factors. This
57 document is a revision of the guideline EMEA/CHMP/BMWP/14327/2006 on the basis of experience
58 from marketing authorisation applications and other new information. It contains, among others, more
59 specific requirements for assays for immunogenicity and integrated analysis of the clinical significance
60 of immunogenicity. The risk of immunogenicity varies between products and product categories, on
61 one hand, and between individuals and patient groups, on the other hand. In order to facilitate the risk
62 analysis, the guideline contains a list of issues to be considered, a multidisciplinary summary of
63 immunogenicity, including risk assessment. This summary allows a risk-based approach to
64 immunogenicity which means that the extent and type of pre-authorisation immunogenicity studies
65 and post-marketing risk management program are tailored according to the risk of immunogenicity
66 and the severity of its potential or observed consequences.

67 From a regulatory point of view, the predictive value of non-clinical studies for evaluation of
68 immunogenicity of a biological medicinal product in humans is low due to differences between human
69 and animal immune systems and to immunogenicity of human proteins in animals. While non-clinical
70 studies aimed at predicting immunogenicity in humans are normally not required, novel models may,
71 for example, be of value in selecting lead compounds for development and unravelling cellular
72 mechanisms.

73 The development of adequate screening and confirmatory assays to measure immune responses
74 against a therapeutic protein is the basis of the evaluation of immunogenicity. Assays capable of
75 distinguishing neutralizing from non-neutralizing antibodies are normally needed. Assays that are used
76 in pivotal clinical trials as well as in post-authorisation studies need to be validated.

77 In the clinical setting, the investigation of immunogenicity should be based on integrated analysis of
78 immunological, pharmacokinetic and clinical efficacy and safety data in order to understand the clinical
79 consequences. The sampling schedule for each product should be determined on a case-by-case basis,
80 taking into account the potential risks associated with unwanted immune responses to patients and the
81 timing of pharmacokinetic and clinical evaluations. Immunogenicity issues should be further addressed
82 in the Risk Management Plan (RMP).

83 Considering the scope of this guideline is wide, the recommendations might have to be adapted on a
84 case-by-case basis to fit into an individual development program. Applicants should consider the
85 possibility to seek Scientific Advice from EMA or from National Competent Authorities.

86 The planning and evaluation of immunogenicity is a multidisciplinary exercise. Therefore, it is
87 recommended that the Applicant will present an integrated summary of the immunogenicity program,
88 including a risk assessment to justify the selected approach.

89 **1. Introduction**

90 Most biological/biotechnology-derived proteins induce an immune response. This immunological
91 response to therapeutic proteins is complex and, in addition to antibody formation, T cell activation or
92 innate immune responses could contribute to potential adverse effects.

93 The consequences of an immune reaction to a therapeutic protein range from transient appearance of
94 antibodies (anti-drug antibodies, ADAs) without any clinical significance to severe life-threatening

95 conditions. Potential clinical consequences of an unwanted immune response include loss of efficacy of
96 the therapeutic protein, serious acute immune effects such as anaphylaxis, and, for therapeutic
97 proteins used for substitution, cross-reactivity with the endogenous counterpart.

98 Many patient-, disease-and product-related factors may influence the immunogenicity of therapeutic
99 proteins. Patient-related factors that might predispose an individual to an immune response include the
100 genetic background, pre-existing immunity, immune status, including immunomodulating therapy, as
101 well as dosing schedule and route of administration. Product-related factors that influence the
102 likelihood of an immune response include the manufacturing process, formulation, and stability
103 characteristics.

104 Although data on possible unwanted immune reactions to therapeutic proteins are required before
105 marketing authorisation, problems may still be encountered in the post-authorisation period.
106 Depending on the immunogenic potential of the therapeutic protein and the rarity of the disease, the
107 extent of immunogenicity data before approval might be limited. Controlled clinical trials cannot be
108 used to study rare adverse effects. Thus, further systematic immunogenicity testing is often necessary
109 after marketing authorization, and may be included in the risk management plan.

110 **2. Scope**

111 The general principles adopted and explained in this document mainly apply to the development of an
112 unwanted immune response against a therapeutic protein in patients and to a systematic evaluation of
113 it. The guideline applies to proteins and polypeptides, their derivatives, and products of which they are
114 components, e.g. conjugates. These proteins and polypeptides are mainly produced by recombinant or
115 non-recombinant expression systems. Throughout this guideline, the term “therapeutic protein” is
116 used.

117 For coagulation factors, please, refer to the specific CHMP guidelines in this area (see chapter 3).

118 **3. Legal basis and relevant guidelines**

119 This guideline has to be read in conjunction with the introduction and general principles (4) and parts
120 II and III of the Annex I to Directive 2001/83 as amended. This guideline should be read in
121 conjunction with other relevant guidelines, e.g.:

- 122 • Guideline on similar biological medicinal products containing biotechnology-derived proteins as
123 active substance: non-clinical and clinical issues (EMA/CHMP/BMWP/42832/2005 Rev. 1)
- 124 • Guideline on Comparability of biotechnology-derived medicinal products after a change in the
125 manufacturing process - non-clinical and clinical issues (EMA/CHMP/BMWP/101695/2006)
- 126 • Guideline on Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical
127 use (EMA/CHMP/BMWP/86289/2010)
- 128 • Guideline on bioanalytical method validation (EMA/CHMP/EWP/192217/2009 Rev.1 Corr.*)
- 129 • Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII
130 products (EMA/CHMP/BPWP/144533/2009)
- 131 • Clinical investigation of recombinant and human plasma-derived factor IX products
132 (superseded by EMA/CHMP/BPWP/144552/2009 rev 1)
- 133 • ICH S6 (R1) Harmonised Tripartite Guideline on Preclinical Safety Evaluation of Biotechnology-
134 Derived Pharmaceuticals

- 135 • ICH Harmonised Tripartite Guideline on Comparability of Biotechnological/Biological Products
136 Subject to Changes in their Manufacturing Process Q5E, 2004

137 **4. Factors that may influence the development of an immune** 138 **response against a therapeutic protein**

139 **4.1. Patient- and disease-related factors**

140 Patient-related factors, which might influence the immune response to a therapeutic protein, include
141 genetic factors, age of the patient, disease-related factors including other treatments, and pre-existing
142 antibodies (protein therapeutic -reactive antibodies) due to previous exposure to the product or
143 products containing substances with structural similarity; as well as sensitisation of patients due to
144 process-and product-related impurities and excipients.

- 145 • *Genetic factors modulating the immune response*

146 Genetic factors may alter immune responses to a therapeutic protein and lead to inter-patient
147 variability. Genetic variation at the level of MHC molecules- and T-cell receptor will modify the immune
148 recognition whereas genetic variation at the level of the modulating factors, such as cytokines and
149 cytokine receptors, may influence the intensity of the response.

- 150 • *Genetic factors related to a gene defect*

151 When the therapeutic protein is used for substitution of an endogenous protein (e.g. factor VIII;
152 enzyme replacement) where the patient is deficient of the natural counterpart, the physiological
153 antigen may represent a neo-antigen and the immune system will interpret the therapeutic protein as
154 foreign or non-self.

- 155 • *Age*

156 Data on immunogenicity from one age group cannot necessarily be projected to others, since immune
157 response to therapeutic proteins can be affected by patient age. Among children, different levels of
158 maturation of the immune system are seen depending on age, and discrepant immune responses to a
159 biological product may be expected.

160 If the product is indicated for children, clinical studies are usually expected to be carried out in this age
161 group. In that case, immunogenicity data should be gathered in these studies as well. If indicated for
162 elderly, consideration should be given to a potentially altered immune response, including
163 autoimmunity.

- 164 • *Disease-related factors*

165 A patient's underlying disease can be an important factor in the context of developing an unwanted
166 immune response. Patients with activated immune systems (for example those suffering from chronic
167 infections, allergies and autoimmune diseases may be more prone to immune responses to therapeutic
168 proteins. In other conditions (e.g. malnutrition, advanced malignant disease, advanced HIV disease,
169 organ failure), an immune response might be less likely to occur due to an impaired immune system.

170 For some products, it has been reported that the development of an antibody response can be different
171 for different therapeutic indications or different stages of the disease. In principle, immunogenicity
172 needs to be addressed in all clinical indications unless justified.

- 173 • *Concomitant treatment*

174 Concomitant therapies may either decrease or increase the risk of an immune response to a
175 therapeutic protein. Typically, the immune reaction against a therapeutic protein is reduced when
176 immunosuppressive agents are used concomitantly. However, an immune response against a
177 therapeutic product is a result of many factors and thus, conclusions on potential impact of the
178 concomitant immuno-modulating medication are not straightforward.

179 Consideration should also be given to previous treatments that can influence the immune reaction to a
180 therapeutic protein and may have a long-term impact on the immune system. If clinical trials of a
181 product with a new active substance are performed in combination with immuno-suppressants, a claim
182 for use of the protein drug in monotherapy must be accompanied by adequate clinical data on the
183 immunogenicity profile in the absence of immuno-suppressive agents.

184 • *Posology-related factors*

185 Factors which may increase the immune response to a therapeutic protein include dosage, dosing
186 schedule and route of administration. Products given intravenously may be less immunogenic than
187 drugs given subcutaneously or intramuscularly. Inhalational and intradermal administration may also
188 enhance immune responses towards the therapeutic protein.

189 Short-term treatment is usually less likely to be associated with a harmful immune response than long-
190 term treatment, and products given continuously are usually less immunogenic than those given
191 intermittently. Intermittent treatment or re-exposure after a long treatment-free interval may be
192 associated with an enhanced immune response.

193 ADA formation against protein therapeutics can be either transient (disappear over time) or sustained.

194 • *Pre-existing antibodies*

195 Previous exposure to similar or related proteins can lead to pre-sensitisation that may modify the
196 response to the new therapeutic protein, e.g. proteins being used for replacement therapy. In addition,
197 sensitisation to excipients in the formulation, as well as to impurities/contaminants from the
198 manufacturing process, may also lead to the generation of pre-existing immunogenicity to the product.

199 Pre-existing reactivity towards the therapeutic proteins (cross-reacting ADAs, rheumatoid factors,
200 antibodies to non-human carbohydrate moieties etc.) at baseline may influence the production of
201 ADAs. Pre-existing antibodies against a variety of protein therapeutics (e.g. monoclonal antibodies,
202 fusion proteins) are frequently encountered, especially in the context of autoimmune diseases. Pre-
203 existing antibodies may also be found in treatment-naïve patients. While the impact of pre-existing
204 antibodies on safety and/or efficacy of biologics is poorly understood, consequences could be severe
205 for e.g. patients receiving 'replacement products' like blood clotting factors, if the previous antibodies
206 are cross-reacting with the newly introduced protein product. Therefore, potential cross-reactivity with
207 pre-existing antibodies should be considered.

208 **4.2. Product related risk factors**

209 Product-related factors influencing the immunogenicity of biotechnology-derived therapeutic proteins
210 include the origin (for instance, the expression construct) and nature of the active substance
211 (structural homology, post-translational modifications), major modifications of the therapeutic protein
212 (e.g. pegylation, fusion proteins, bispecific antibodies, conjugates between a protein and a chemical
213 drug/moiety), product-related (e.g. degradation products, impurities, aggregates) and process-related
214 impurities (host cell proteins, lipids or DNA, bacterial contaminants), formulation (excipients) and
215 product packaging (containers, stoppers).

216 • *Protein structure and immunological tolerance*

217 T cell-dependent antibody responses involve a complex interplay among antigen presenting cells, T
218 cells, secreted cytokines and B cells, subsequently leading to B cell activation, somatic hypermutation
219 and antibody production and sometimes to immunoglobulin class-switch. Within this cascade, there are
220 central and peripheral immunological factors that together form the basis of immunological tolerance
221 to endogenous proteins.

222 Immune tolerance to endogenous proteins is variable; in general tolerance is weaker for low-
223 abundance proteins than for high-abundance proteins. Thus, levels of cytokines and growth factors are
224 low whereas autoantibodies towards cytokines and growth factors in healthy individuals are not
225 uncommon.

226 Biotechnology-derived analogues to human endogenous proteins may trigger an immune response due
227 to variations in the amino acid sequence or changes to the protein structure as a result of post-
228 translational modifications or other changes during all steps of the manufacturing process, storage and
229 administration.

230 The key driver of mature immunological reactions is the activation of T cells. T cell epitopes are linear
231 peptides. Thus, a difference in the amino acid sequence between an endogenous and a therapeutic
232 protein may modify T cell epitopes.

233 T cell-independent antibody responses may be generated when B cells recognise a repeated pattern in
234 the biological product (polysaccharides, lipopolysaccharides, possible moieties in the therapeutic
235 protein, aggregates), that elicits low-affinity IgM antibodies. However, switching to IgG classes
236 including clonal expansion, may also take place, and the mechanism behind this evolution of the
237 immune reaction is still not fully understood.

238 Glycosylation can influence both the physico-chemical and biological properties of a protein. The
239 presence and structure of carbohydrate moieties may have both a direct or indirect impact on the
240 immunogenicity of therapeutic proteins; the glycan can induce an immune response itself (e.g. glycans
241 of non-human origin), or its presence may affect the conformation of the protein in such a way that
242 the protein becomes immunogenic.

243 Fusion proteins may contain neo-epitopes due to the introduction of foreign peptide sequences, e.g. in
244 linkages/fusion junctions. Antibodies generated specifically against the polyethylene-glycol part of
245 pegylated proteins have been identified. However, pegylation and glycosylation may also decrease
246 immunogenicity by shielding the immunogenic epitopes, while maintaining the native conformation of
247 the protein.

248 Fusion proteins composed of a foreign and self-protein may be of concern, especially because of the
249 potential of the foreign moiety to provoke an immune response to the self-protein (epitope spreading).
250 Thus, identification of the antigenic moiety of a fusion protein is useful.

251 • *Formulation and packaging*

252 The composition of a formulation is chosen in order to increase the stability of the product, i.e. to best
253 maintain the native conformation of therapeutic proteins. A successful, robust formulation depends on
254 the understanding of the physical and chemical nature of the active substance and the excipients alone
255 and their interaction. The formulation and the source of excipients may influence the immunogenicity
256 of therapeutic proteins. This should be taken into account when introducing variations to the
257 formulation.

258 In addition, interactions between the protein substance, excipients in the chosen formulation, and the
259 primary packaging (e.g. leachables and impurities from stoppers and syringes derived from their
260 manufacturing process; like tungsten) as well as the conditions for clinical use (e.g. dilution of infusion
261 solutions and infusion devices of different materials), may impact on product quality and generate
262 negative effects like adherence to walls, denaturation and aggregation. Both denaturation and
263 aggregation of the protein therapeutic may potentially trigger an immune response.

264 • *Aggregation and adduct formation*

265 Aggregation and adduct formation of proteins may either reveal new epitopes or lead to the formation
266 of multivalent epitopes, which may stimulate the immune system. Aggregation can enhance a protein-
267 specific immune response and lead to the formation of ADAs. Removal of aggregates (present as
268 visible or sub-visible particles) has been associated with strongly reduced immunogenicity in preclinical
269 in vivo studies.

270 Factors which may contribute to aggregate or adduct formation include formulation, purification
271 processes, viral inactivation procedures (low pH), packaging material and storage conditions of
272 intermediates and finished product. The use of proteins as excipient, e.g. albumin, may lead to the
273 formation of more immunogenic aggregates. It is important to monitor the aggregate and adduct
274 content of a product throughout its shelf life.

275 Higher-molecular weight (MW) aggregates are more prone to elicit immune responses than lower-MW
276 aggregates, and the repetitive ordered epitopes (multimeric epitopes) that are often displayed by
277 protein aggregates (e.g. viral-like arrays) may involve T cell independent mechanisms and activate B
278 cells directly. Extensive crosslinking of B cell receptors by higher order structures can activate B cells
279 to proliferate and produce antibodies not only to the aggregated, but also to the monomeric form of
280 the protein.

281 • *Impurities*

282 There are a number of potential impurities in the drug substance of therapeutic proteins, which
283 potentially can serve as adjuvants. Host cell proteins from the cell substrate co-purified with the active
284 substance, could induce immune responses against themselves, as well as to the active substance.
285 Bacterial proteins, contaminants from the manufacturing process, host cell-derived lipids or DNA could
286 also function as adjuvants triggering immune responses against the therapeutic protein.

287 **5. Potential clinical consequences of immunogenicity**

288 Immunogenicity of a protein therapeutic may have profound effects on the efficacy and safety of the
289 product. Factors which determine whether antibodies to a therapeutic protein will have clinical
290 significance include the epitope recognised by the antibody, the affinity and class of the antibody, the
291 amount of antibodies generated, as well as the pharmacological properties of the biotechnological
292 medicinal product. In addition, the ability of immune complexes to activate complement may have an
293 impact on the clinical outcome.

294 **5.1. Consequences on Efficacy**

295 ADAs can influence efficacy by eliminating the pharmacological action of the product or altering its
296 pharmacokinetic profile.

297 “Neutralising” antibodies can cause a reduction or loss of efficacy by binding to or near the active site,
298 or by inducing conformational changes. Usually, ‘non-neutralising’ antibodies are expected to be

299 associated with less clinical consequences. However, as discussed below, such antibodies may reduce
300 the exposure to the therapeutic protein and thereby influence efficacy indirectly.

301 The effects of ADAs on therapeutic proteins may vary from zero to complete loss of efficacy.
302 Sometimes, the efficacy is reduced gradually over time without a clear correlation to ADA titres.

303 **5.2. Consequences on Safety**

304 In general, most adverse effects of therapeutic proteins are related to their pharmacological effects.
305 The main exception is their potential immunogenicity. Immune-based adverse effects may be both
306 acute and delayed.

307 Less severe immune-based adverse effects include infusion-site reactions. Non-allergic (not involving
308 IgE-generation) infusion reactions are typically seen during the first infusions and can be mitigated by
309 appropriate pre-medication.

- 310 • *Hyper acute / acute reactions*

311 Acute infusion-related reactions including anaphylactic / anaphylactoid reactions (type I), may develop
312 within seconds or during a few hours following infusion.

313 All infusion-related reactions involve the immune system; however, some (anaphylactic) are allergic in
314 nature and are usually mediated by immunoglobulin E (IgE), whereas others (anaphylactoid) are not
315 true allergic reactions and are not mediated by IgE. Although infusion-related reactions can be allergic
316 or non-allergic, the clinical manifestations are the same. Acute reactions can cause severe
317 hypotension, bronchospasm, laryngeal or pharyngeal oedema, wheezing and/or urticaria. The term
318 anaphylaxis should be restricted to such situations and represent a strict contraindication to further
319 exposure to the drug.

320 Usually, patients who develop antibodies are more prone to suffer from infusion-related reactions.

321 A thorough assessment concerning a products' potential to inducing hyper acute / acute infusion –
322 related reactions, as well as the identification of all cases meeting the clinical diagnostic criteria for
323 anaphylaxis regardless of presumed pathophysiology, is important.

- 324 • *Delayed reactions*

325 In addition to acute reactions, delayed type (T cell mediated) hypersensitivity and immune complex-
326 mediated reactions have to be considered. The risk of such reactions may be higher with an increasing
327 drug free interval. Delayed hypersensitivity reactions should be clearly delineated from infusion-related
328 reactions. Applicants should ensure the systematic collection of non-acute clinical sequelae following
329 application of the therapeutic protein. Clinical signs can include myalgia, arthralgia with fever, skin
330 rash, and pruritus, but other, less obvious clinical symptoms should be systematically collected as well.

- 331 • *Autoimmunity: Cross-reactivity to an endogenous counterpart*

332 A possible life-threatening clinical consequence of ADA formation against a therapeutic protein is cross-
333 reactivity with an endogenous protein when this protein has a non-redundant role in key physiological
334 functions. For example, ADAs cross-reacting with endogenous erythropoietin have caused pure red cell
335 anaemia in epoetin alfa-treated patients with kidney failure. Novel constructs, like hybrid molecules
336 fused to physiological functional molecules, should be carefully investigated for ADAs cross-reacting
337 with relevant endogenous (or self) proteins.

338 **6. Non-clinical assessment of immunogenicity and its**
339 **consequences**

340 Therapeutic proteins show species differences in most cases. Thus, human(ised) proteins will be
341 recognised as foreign proteins by animals. For this reason, the predictivity of non-clinical studies for
342 evaluation of immunogenicity in humans is considered low. Non-clinical studies aiming at predicting
343 immunogenicity in humans are normally not required.

344 However, ongoing consideration should be given to the use of emerging technologies (novel *in vivo*, *in*
345 *vitro* and *in silico* models), which might be used as tools during development or for a first estimation of
346 risk for clinical immunogenicity. *In vitro* assays based on innate and adaptive immune cells could be
347 helpful in revealing cell-mediated responses.

348 It is expected that (non-)clinical studies are supplied with material sufficiently representative of the
349 medicinal product that is going to be placed on the market. Since immunogenicity concerns may arise
350 from the presence of impurities or contaminants, it is preferable to rely on purification processes to
351 remove impurities and contaminants rather than to establish a preclinical testing program for their
352 qualification (refer to ICH S6 (R1) Harmonised Tripartite Guideline on Preclinical Safety Evaluation of
353 Biotechnology-Derived Pharmaceuticals).

354 Measurement of anti-drug antibodies in non-clinical studies may be needed as part of repeated dose
355 toxicity studies, in order to aid in the interpretation of these studies (as discussed in "ICH S6 (R1)
356 Harmonised Tripartite Guideline on preclinical safety evaluation of biotechnology-derived
357 pharmaceuticals."). Blood samples should be taken and stored for future evaluations if then needed.
358 The assays used should be validated (see also chapter 7.3). In toxicology studies, where usually higher
359 concentrations of therapeutic protein are present in the samples, the interference of the therapeutic
360 protein in the ADA assays needs to be considered.

361 In the development of similar biological medicinal products (biosimilars), the comparison of the anti-
362 drug antibody response to the biosimilar and the reference product in an animal model is not
363 recommended as part of the biosimilar comparability exercise, due to the low predictivity for the
364 immunogenicity potential in humans.

365 An immune response to a therapeutic protein representing a counterpart to an endogenous protein
366 may result in cross-reactivity, directed to the endogenous protein in cases where endogenous protein
367 is still produced. Any relevant experience on the consequences of induction of an immune response to
368 the endogenous protein or its absence/dysfunction in animal models should be discussed in the
369 integrated summary of immunogenicity. Both humoral and cellular immune responses (where relevant)
370 should be considered. Usually, safety risks would be predictable, based on existing knowledge on the
371 biological functions of the endogenous protein and animal studies would not be required to confirm
372 these safety risks. Only in absence of sufficient knowledge, and if theoretical considerations are
373 suggestive of a safety risk, animal immunisation studies with the therapeutic protein or the animal
374 homolog may be considered in order to gain information on the potential consequences of an unwanted
375 immune response.

376

377

378 **7. Development of assays for detecting and measuring** 379 **immune responses in humans**

380 Developing an integrated analysis strategy relevant for the intended treatment plan is critical for
381 elucidating the clinical relevance of immunogenicity data. It is very important to select and/or develop
382 assays and assay strategies for assessment of immune responses. While most effort is usually focused
383 on antibody detection and characterisation as this is often related to clinical safety and efficacy, cell-
384 mediated responses are also important and their assessment may be considered by applicants on a
385 case by case basis.

386 Although assays will be refined during product development and assay suitability reassessed as per
387 their use, the applicant is expected to provide data supporting full assay validation for marketing
388 authorisation.

389 **7.1. Strategy and Antibody Assays**

390 Adopting an appropriate strategy which includes use of sensitive and valid methods for immunogenicity
391 assessment is essential. Typically, a multi-tiered approach should be employed. This includes a
392 screening assay for identification of antibody positive samples/patients, a procedure for confirming the
393 presence of antibodies and determining antibody specificity followed by functional assays for the
394 assessment of the neutralizing capacity of antibodies. Tests for determining antibody isotype and
395 epitope specificity may also be considered on confirmed antibody positive samples. In some cases,
396 testing samples for cross-reactivity with other products based on the same protein and the
397 endogenous protein is important as it may have implications for clinical efficacy and safety.

398 In addition, assays for measuring the level of the product and for assessing clinical relevance to
399 products e.g., assays for relevant biomarkers or pharmacokinetic measurements are required to
400 evaluate the clinical impact of induced antibodies if these are detected (see Annex 1).

401 Evaluation of the kinetics of antibody development and the duration as well as the magnitude of the
402 antibody response is important as it may correlate with clinical consequences.

403 If antibodies are induced in patients, serum or plasma samples need to be characterised in terms of
404 antibody level (titre), neutralizing capacity and possibly other criteria determined on a case-by-case
405 basis according to the biological product, the type of patients treated, the aim of the study, clinical
406 symptoms and possibly other factors. These may include antibody class and subclass (isotype), affinity
407 and specificity. The degree of characterization required will differ depending on the study purpose and
408 stage of development of the product. The assays used should be qualified for their intended purpose.

409 • *Screening assays*

410 Screening assays are the first step in immunogenicity evaluation. They should be sensitive and capable
411 of detecting all antibodies (including IgM and IgG subclasses) induced against the product in all
412 antibody positive patients. A low false positive rate is desirable but false negative results are
413 unacceptable.

414 Screening is performed using immunoassays which are based on a variety of formats and detection
415 systems. All screening procedures detect antigen-antibody interaction (binding) but may differ in their
416 underlying scientific/ technical principles. These assays are configured to have moderate throughput
417 and appropriate automation, however each assay has its own attributes and inherent limitations which
418 need to be considered (see chapter 10).

419 Assays need to be developed, optimized and selected according to and taking account of their intended
420 use. The importance and requirements of assay characteristics depends on the use of the assay.
421 Adoption of the simplest assay suitable for all requirements is normally a valid approach to assay
422 selection. However, care with this is necessary to ensure that it does not compromise other stages of
423 immunogenicity assessment. For example direct binding ELISAs, with antigen directly immobilized on
424 plate well surfaces are often the simplest assay approach, but may be associated with a very high
425 incidence of false positivity. They may also be associated with a high incidence of false negatives for
426 samples containing low affinity antibodies. It is often necessary to adopt a more suitable assay, e.g.
427 bridging assays, electrochemiluminescence or surface plasmon resonance methods. Epitope masking
428 can give false negative results in screening assays and a strategy to avoid this may be necessary e.g.
429 by labelling detecting reagents using procedures that avoid masking of particular epitope(s).

430 In this respect the reagents (e.g. blocking reagents) should be considered carefully. Blocking reagents
431 like BSA and milk contain non-human glycans that are sometimes found on proteins produced in non-
432 human animal cells. Thus, antibodies against these glycans may be missed.

433 Samples (normally serum or plasma) may contain substances that interfere with the assays i.e. matrix
434 effects which produce false positive or negative results and/or incorrect assessment of antibody
435 content. Examples include complement components or complement receptors, mannose binding
436 protein, Fc receptors, soluble target molecules, and rheumatoid factors. The influence of such matrix
437 components on assay results should be considered and measured. To mitigate the potential influence
438 corrective measures should be implemented. Applicants need to justify the suitability of the chosen
439 approach, taking into consideration the limitations of the respective methods.

440 Additionally, residual therapeutic product present in patients' blood can complex with induced antibody
441 and hence reduce the amount of antibody detectable by assays. This may affect assays differently,
442 depending on the assay, assay format or type and the antibody characteristics. If this occurs, it may
443 be circumvented/resolved by using a number of approaches e.g. by dissociating the immune-
444 complexes with acid, removing excess biological by solid-phase adsorption, use of long incubation
445 times and/or using an assay which allows sufficient sample dilution to avoid this problem. Such
446 approaches must themselves be validated for effectiveness and adopted on a case-by-case basis
447 according to needs. In some cases this problem can be overcome by appropriate spacing of the timing
448 between administration of product and sampling for antibody assessment i.e. allowing time for the
449 product to be cleared from the circulation before sampling. However this latter approach must not
450 significantly compromise the detection of antibodies or the treatment of the patient. In any case, the
451 Applicant has to demonstrate that the drug-tolerance of the assay exceeds the levels of the therapeutic
452 protein in the samples for ADA testing.

453 • *Assays for confirming the presence of antibodies*

454 Confirmatory assays are necessary for eliminating any false positive results following the initial screen.
455 Assay selection should take account of the limitations and characteristics of the screening assay. A
456 common approach for confirming antibodies is addition of an excess of antigen to the sample followed
457 by a comparison of spiked and unspiked sample in the binding assay. This should result in a reduction
458 of positive signal for true positives in the spiked sample.

459 Antibodies present in confirmed positive samples need to be examined for specificity for the active
460 protein and, in relevant cases, distinguished from antibodies which bind to product-related and
461 process-related components (e.g., host cell proteins). It has been shown that antibodies can be
462 induced against all or any of these.

463

464 • *Neutralization assays*

465 Determination of the neutralizing potential of the induced antibodies is an essential element of
466 immunogenicity assessment. Deviation from this concept needs a strong justification. Neutralizing
467 antibodies (NAbs) inhibit the biological activity of a therapeutic by binding to epitope(s) within or close
468 to the active site(s) of the molecule or by causing conformational changes. Because NAbs can trigger
469 clinical effects, specific and sensitive *in vitro* methods are needed for detection. Two types of NAb
470 assays are mainly used - cell-based and non-cell-based assays.

471 An assay must be selected or developed which responds well to the biological product. Bioassays used
472 for potency estimation can often be adapted to assess neutralising antibodies. However, they
473 frequently require refining if they are to perform optimally for measuring the neutralizing capacity of
474 antibodies.

475 Understanding the mode of action, the target and effector pathways of the therapeutic are critical for
476 identification of a suitable NAb assay format. Additionally, the risk of developing NAbs and the impact
477 on clinical sequelae also needs to be considered. While cell-based assays are often employed for
478 agonistic therapeutics, non-cell-based CLB assays are often considered for antagonistic molecules with
479 humoral targets. For products that do not have intrinsic biological activity (e.g., some MAbs),
480 competitive ligand binding assays (CLB) or other alternatives may be suitable. However, when these
481 are used it must be demonstrated that they reflect neutralizing capacity/potential in an appropriate
482 manner. For antagonists such as monoclonal antibody therapeutics with effector functions for clinical
483 efficacy, cell-based assays are recommended as the mechanism of action cannot be adequately
484 reflected in a non-cell-based CLB assay.

485 The neutralising capacity of antibodies present in positive samples needs to be established as this often
486 correlates with diminished clinical responses to biological product. Usually a single concentration of
487 biological is chosen for the assay and dilutions of each sample assessed for their inhibitory effect on
488 the assay response. This allows a neutralizing dose response to be determined and calculation of
489 neutralizing capacity ('titre') for each sample

490 As for screening, inclusion of a step confirming that the neutralization is truly related to the antibodies
491 and not due to other inhibitory components in sample matrix is useful. Approaches for showing
492 specificity such as antibody depletion, use of alternative stimuli (if assay responds to multiple stimuli)
493 can be considered.

494 It should be noted that neutralizing activity does not necessarily correlate with binding antibody
495 content, i.e. samples containing significant or high amounts of binding antibodies may fail to neutralize
496 biological activity whereas samples containing lower amounts of binding antibodies can neutralize
497 variable (sample dependent) amounts. This may depend on product, but must be determined
498 empirically.

499 • *Immunogenicity Assessment strategy –design and interpretation*

500 Immunogenicity studies need to be carefully and prospectively designed to ensure all essential
501 procedures are in place before commencement of clinical assessment. This includes the selection,
502 assessment, and characterisation of assays, identification of appropriate sampling points including
503 baseline samples for determination of pre-existing antibodies, adequate sample volumes and sample
504 processing/storage and selection of statistical methods for analysis of data.

505 This applies to assays used to measure and characterise antibodies and to methods employed for
506 assessing clinical responses to antibodies if they are induced. Much of this needs to be established on a
507 case-by case basis, taking account of product, patients, and expected clinical parameters.

508 **7.2. Assay Controls and Reagents**

509 The identification and/or development of appropriate well characterized positive and negative controls
510 are crucial. These reagents are essential for assay calibration and validation. They are intimately
511 associated with assay interpretation and with distinguishing antibody positive from antibody negative
512 samples.

513 Ideally, an antibody positive control should be a human preparation with a significant antibody content
514 which is available in sufficient quantity for continued use. However, sufficient human serum is often
515 not available to serve as a positive control preparation. In such cases, use of an animal serum raised
516 against the product as a reference is the only option. However, its use is more limited than a human
517 preparation e.g. immunochemical procedures, which involve the use of an anti-human immunoglobulin
518 reagent, will not reliably respond to non-human antibodies and the response in all assays may differ in
519 characteristics from responses to human antibodies in human samples.

520 Use of the positive control for estimating antibody levels in binding assays in mass units is
521 problematical as the immunoglobulin present in standards and samples is heterogeneous in structure,
522 specificity and avidity. This makes direct valid comparison between samples and positive control
523 difficult, if not impossible. An option is to report immunoassay data as a titre based on a standard
524 procedure for calculating this value.

525 The positive control antibodies for neutralization assays should have significant neutralizing activity,
526 but it is also useful to include a non-neutralizing antibody preparation in assays, at least in validation
527 studies. Biological assays used to assess the neutralizing capacity of antibodies may be calibrated
528 using International Standards/Reference Preparations where these are available. This would allow
529 expression of neutralizing activity in terms of meaningful units of biological activity of product/
530 preparation and also provide information relevant to assay validation. If such standards are not
531 available, appropriate in-house preparations can be established. In many cases, it is useful to express
532 the neutralizing capacity of samples in terms of the volume of sample required to neutralize a constant
533 biological activity of product e.g. ml of serum/defined unit of bioactivity of biological. Using the sample
534 dilution or titre required to neutralize the biological activity of the product is also an option.

535 It is also very useful to prepare a panel of reference materials containing different amounts of
536 antibodies and antibodies with different characteristics e.g. neutralizing/non-neutralizing, which can be
537 used to characterize/validate assays and act as assay performance indicators. If possible this should
538 include one or more preparations with low antibody content (close to the minimum detection limit) and
539 containing low avidity antibodies.

540 Negative controls are needed to establish assay baselines and characterize/validate the assays. Assay
541 baseline for normal (healthy) individuals is clearly fairly easily determined by measuring the assay
542 response using samples derived from an appropriate number of such individuals and analysing this to
543 provide a statistically valid background value. However, this may not be representative of the baseline
544 response of samples derived from the patient population, which would therefore need to be established
545 separately, using pre-treatment samples from patients or drug naïve disease patients. Some
546 individual's/patient's samples may contain pre-existing (pre-treatment) antibodies or possibly other
547 substances which produce significant positive responses in assays, and so screening patients for this is
548 necessary to ensure that post-treatment data can be interpreted correctly in terms of treatment
549 emergent antibodies.

550 Reagents used in assays need to be qualified and acceptance specifications set, at least for those which
551 are most important. They should be stored appropriately (lyophilized or frozen at a suitable
552 temperature) and characterized.

553 **7.3. Assay validation and interpretation of results**

554 Assay validation is an ongoing process throughout product development. Assays used for the pivotal
555 clinical trials need to be validated for their intended purpose. Validation studies must be conducted to
556 establish that the assays show appropriately linear, concentration dependent responses to relevant
557 analytes as well as appropriate accuracy, precision, sensitivity, specificity and robustness. Inclusion of
558 data supporting minimal required dilution of samples is important. For pivotal clinical trials, the use of
559 a central laboratory to perform the assays is helpful to avoid inter-laboratory variability. In the post-
560 approval setting, it is also important to consider inter-laboratory variability. Assays must also be
561 validated to show that matrix effects caused by reagents or substances present in samples do not
562 adversely affect the results obtained. This is normally addressed by 'recovery' investigations conducted
563 by observing the effects of such substances present in the matrix on the response obtained in their
564 absence. This needs to be investigated for the full range of dilutions of samples, which are to be used
565 in assays, and, at least in some cases, limits the dilutions, which can be validly assessed.

566 It is essential to establish clear criteria for deciding how samples will be considered positive or
567 negative, and also how positive results will be confirmed. Approaches to these can differ according to
568 assay etc. and need to be decided accordingly. A common procedure for establishing positive cut-off
569 for immunoassays is to establish assay background using samples from normal healthy controls and or
570 diseased individuals (see above). A statistical approach should be used to establish the assay cut-off
571 value. Alternatively, real data (e.g. double background value) can be used to determine what will be
572 considered the lowest positive result. For antibody positive samples, a titre needs to be determined
573 using a standard approach and reporting the reciprocal of the highest dilution at which the sample
574 gives a positive result. Another option is to report in mass units using a positive antibody control but
575 this has caveats as explained above.

576 **7.4. Assays for comparative immunogenicity**

577 Comparative immunogenicity studies are always needed in the development of biosimilars (see
578 Guideline on similar biological medicinal products containing biotechnology-derived proteins as active
579 substance: non-clinical and clinical issues, EMEA/CHMP/BMWP/42832/2005 Rev1) but rarely for a
580 change of the manufacturing process of a given biological product (for changes to the manufacturing
581 process of the drug substance see ICH Harmonised Tripartite Guideline on Comparability of
582 Biotechnological/Biological Products Subject to Changes in their Manufacturing Process Q5E, 2004).

583 Immunogenicity testing of the biosimilar and the reference product should be conducted within the
584 biosimilar comparability exercise by using the same assay format and sampling schedule which must
585 meet all current standards. Analytical assays should be performed with both the reference and
586 biosimilar molecule in parallel (in a blinded fashion) to measure the immune response against the
587 product that was received by each patient. The analytical assays should preferably be capable of
588 detecting antibodies against both the biosimilar and the reference molecule but should at least be able
589 to detect all antibodies developed against the biosimilar molecule. Usually, the incidence and nature
590 (e.g. cross-reactivity, target epitopes and neutralising activity) of antibodies and antibody titres should
591 be measured and presented and should be assessed and interpreted in relation to their potential effect
592 on clinical efficacy and safety parameters.

593 When comparative immunogenicity studies are required in the context of a manufacturing change of a
594 given product, assays to compare the pre- and post-change products need to be developed. Ideally,
595 there should be two assays, one using the pre-change protein and the other with the post-change
596 protein as the target antigen.

597 **7.5. Immunogenicity assessment of conjugated proteins and fusion**
598 **proteins**

599 Elicitation of an antibody response with multiple specificities and variable affinity towards different
600 epitopes resulting in varying degrees of clinical impact is expected for novel biotherapeutic molecules
601 such as engineered fusion proteins and chemically conjugated proteins. The evaluation of this
602 response, in particular, the characterization of the specificity of the induced antibodies is challenging
603 and may require multiple assays for measuring immune responses to various moieties. Alternatively, a
604 strategy based on the competitive inhibition principle of the confirmatory assay to dissect the
605 specificities of the antibodies to individual moieties can be used. For example, for a pegylated protein,
606 the assessment strategy would comprise a screening assay using the pegylated therapeutic and testing
607 of any positive samples using the whole therapeutic, the non-pegylated protein and the PEG moiety in
608 a confirmatory assay.

609 **7.6. Characterisation of antibodies to a therapeutic protein**

610 Normally, the incidence and titre, persistence and neutralizing capacity of the ADAs are required. In
611 certain circumstances, it may be feasible to further characterize the ADA response, e.g., in case of
612 anaphylactoid reactions and follow up of the maturity of emerging immune response. In these cases,
613 determination of the isotype and IgG-subclasses or even T cell reactivity may be useful. Cross-
614 reactivity of the ADAs with relevant endogenous proteins should be investigated if emerging
615 autoimmunity is suspected.

616 **8. Immunogenicity and Clinical Development**

617 Testing of immunogenicity should be included in all pivotal clinical trials of a new biological medicinal
618 product targeting patient populations that have not been exposed to the product previously. The aim is
619 not only to demonstrate an immune response to the product but also to investigate correlations
620 between binding and neutralising ADAs, on one hand, and pharmacokinetics and –dynamics as well as
621 efficacy and safety, on the other hand. Therefore, assessment of immunogenicity should be included in
622 the planning of the clinical trials, including the synchronization of sampling for ADAs and relevant
623 biomarkers as well as evaluation of efficacy and safety (see chapter 10).

624 **8.1. Rationale for sampling schedule and kinetics of the antibody response**

625 Immunogenicity should be systematically tested in patients by scheduled routine repetitive sampling as
626 well as in a symptom-driven manner with additional samples, when the occurrence of an unwanted
627 immune response is suspected.

628 Several product-related factors will influence the development of an immune response against a
629 therapeutic protein (see chapter 4). Therefore, the sampling schedule for detection of an immune
630 response should be adapted and selected individually for each product, also taking into account its
631 pharmacokinetics (e.g. elimination half-life) and the drug tolerance of the ADA-assay(s). Baseline
632 samples should always be collected.

633 Applicants should endeavour to standardise, assays as well as terminology and definitions of potential
634 immune-mediated adverse effects taking into account also experience with comparable products and
635 relevant regulatory and scientific publications (see also chapter 10). During treatment, samples should
636 also be taken before administration of the product, since residual levels of the active substance in
637 plasma can interfere with the assay (see chapter 7).

638 The frequency of sampling and the timing and extent of analyses will also depend on the risk
639 assessment for a particular drug (as described in the integrated summary of immunogenicity, chapter
640 10). Sampling schedules should be designed to distinguish patients being transiently positive from
641 patients developing a persistent antibody response. The post-treatment follow up sampling should be
642 long enough to allow conclusions on the persistence of the immune response triggered by the
643 therapeutic protein and uncover an immune reaction that was suppressed by the therapeutic protein
644 itself. The timing of post-treatment sample(s) is determined by the half-life of the protein and the drug
645 tolerance of the ADA assay. The first post-treatment sample should not be taken earlier than four
646 weeks after the last dose.

647 More frequent sampling is necessary in the earlier phase of treatment, where patients are normally
648 most at risk of antibody development. Since longer-term treatment is more likely to result in an
649 immune response, routine, less frequent sampling later in the treatment course should be
650 implemented in clinical trials. In case of continuous chronic treatment, immunogenicity data for one
651 year of treatment should become available pre-authorisation but shorter follow up is possible with a
652 proper justification.

653 The immunogenicity associated with intermittent treatment should be considered on the basis of a risk
654 assessment, e.g. experience from other similar products, risks associated with potential
655 immunogenicity, persistence or appearance of antibodies after the exposure.

656 If used for different routes of administration, Applicants should justify their approach as regards
657 immunogenicity assessment for each route at the time of Marketing Authorisation Application (see
658 integrated summary on immunogenicity).

659 The risk of immunogenicity and its possible consequences should be described in the relevant chapters
660 of the SmPC in a concise way and taking account the fact that a comparison of results from different
661 sources and or by different assays is unreliable. The feasibility of and possibilities for routine
662 monitoring of immunogenicity, including the usefulness of drug concentration measurements, should
663 also be included in the SmPC, if applicable.

664 **8.2. Consequences on pharmacokinetics of the product**

665 Antibodies recognising epitopes outside the active sites of the protein (non-neutralising) may be
666 associated with fewer clinical consequences than the neutralising antibodies. However, such antibodies
667 can influence pharmacokinetics, especially the elimination phase. Non-neutralizing, "binding"
668 antibodies, may sometimes also increase, rather than decrease, the efficacy of a product by prolonging
669 the half-life, or stimulating a pathway or mechanism. A change in pharmacokinetics may be an early
670 indication of antibody formation. Thus, the Applicants are encouraged to incorporate concomitant
671 sampling for both pharmacokinetics and immunogenicity into all repeat dose studies.

672 **8.3. Impact of immunogenicity on safety and efficacy**

673 The presence of ADAs may or may not have clinical consequences. It is essential that the clinical
674 development is based on an analysis of potential risks and possibilities to detect and mitigate them.
675 The planning of the analysis of immune-mediated adverse effects should be based on risk analysis,
676 including previous experience of the product (class), presence of potentially immunogenic structures in
677 the protein and patient population (see integrated summary). Patients with pre-existing antibodies
678 may exhibit a different safety profile and should be analysed as a subgroup. The analysis plan should
679 define symptom complexes that might be associated with acute or delayed hypersensitivity and

680 autoimmunity as well as with the loss of efficacy (see chapter 10). Potential immunological adverse
681 effects should be addressed in the risk management plan (see chapter 9).

682 When ADAs have been demonstrated, further characterization beyond the titre and neutralizing
683 capacity of the antibodies may be useful, e.g. immunoglobulin class in case of acute hypersensitivity. It
684 may also be possible to determine a “threshold” level of ADAs beyond which there is a significant
685 impact on efficacy and/or safety.

686 ***8.4. Methodological aspects to assess comparability of immunogenicity*** 687 ***potential as part of a comparability exercise***

688 Comparative immunogenicity studies are always required in the development biosimilar products
689 (Similar biological medicinal products containing biotechnology-derived proteins as active substance:
690 non-clinical and clinical issues, EMEA/CHMP/BMWP/42832/2005 Rev. 1) and occasionally after changes
691 to the manufacturing process of a given product, before or after marketing authorisation. When
692 changes to the manufacturing process of a licensed product are made, the comparability exercise is a
693 stepwise process (see ICH Q5E). If the initial physicochemical and biological testing indicates a
694 difference between the pre- and post-change versions of the product, the potential consequences to
695 safety and efficacy need to be considered, including altered immunogenicity.

696 The type of immunogenicity studies, if required, should be justified on the basis of the observed
697 difference(s), route of administration, dose-response curve and therapeutic window, the potential
698 clinical impact, and knowledge gained with this product and product class before (see ICH Q5E). The
699 target population needs to be sensitive for differences in immunogenicity and its consequences and be
700 representative for the population(s) for whom the product is indicated. In high risk situations, the
701 samples should be analysed on ongoing basis.

702 In the cases when a manufacturing process change needs to be supported by a clinical trial,
703 investigation of immunogenicity should be integrated with the pharmacokinetic, safety, and efficacy
704 testing. Immunogenicity evaluation as part of a clinical trial for a comparability exercise in the context
705 of a manufacturing change should preferably involve head-to-head study of pre- and post-change
706 product.

707 Increased immunogenicity as a result of a change in the manufacturing process will question the
708 comparability. If the observed difference is uncertain, a specific risk management strategy and an
709 update of the risk management plan (see chapter 9) may be required. If there is a risk of rare
710 immune-mediated adverse effects, this may be addressed after the implementation of the change in a
711 post-marketing setting. Decreased immunogenicity should be discussed and justified, including
712 potential root cause and impact on exposure.

713 ***8.5. Management of immunogenicity***

714 The presence of an immune reaction to a therapeutic protein may lead to clinical consequences in spite
715 of the efforts by the Applicants to select compounds that have a reduced immunogenic potential (see
716 chapter 6). In this case, the Applicant needs to explore possibilities to reduce the adverse impact of
717 immunogenicity observed during the clinical development.

718 Applicants should provide guidance to the prescriber as part of the Summary of Product Characteristics
719 on how to mitigate the effects of immunogenicity. An evidence based recommendation is needed to
720 guide prescriber as when to stop the treatment of a patient with loss of efficacy or side effects or when
721 an immunosuppressive co-medication, an increase of dose or a reduced dosing interval might be
722 helpful. In some cases, as with coagulation factors, it may be possible to re-establish the

723 immunological tolerance by tolerisation, e.g. by administration of large doses of the therapeutic protein
724 or normal intravenous immunoglobulin. Certain hypersensitivity symptoms may be prevented or
725 mitigated by appropriate pre-medication.

726 **9. Pharmacovigilance**

727 Within the authorisation procedure, the applicant should present a risk management plan (RMP) in
728 accordance with current EU legislation and pharmacovigilance guidelines. Immunogenicity should
729 always be considered in the safety specification section of the RMP of biotechnology-derived
730 therapeutic proteins and the need for additional pharmacovigilance activities should be evaluated. For
731 changes in the manufacturing process, implications of this change on the immunogenic potential also
732 have to be addressed in the RMP. Again, it should be emphasized that evaluation of immunogenicity is
733 a multidisciplinary approach, at best providing input of quality, non-clinical and clinical experts.

734 The extent of data on immunogenicity that can be obtained during the clinical development program of
735 a biotechnology-derived product before approval depends on the event rate, driven both by the
736 immunogenic potential of the protein and the rarity of the disease. The availability of data on
737 immunogenicity at time of approval might, therefore, be limited. In addition, knowledge obtained for
738 the product class and/ or the reference product (in the case of biosimilar development) should be
739 discussed in the RMP. The potential for immunogenicity should be fully evaluated based on the
740 available evidence with appropriate conclusions drawn on whether or not a product may pose such a
741 (potential) risk. If this is the case, immunogenicity should be included in the RMP as either a potential
742 or identified risk. Immunogenicity should always be related to the clinical consequence, e.g. drug
743 neutralizing antibodies resulting in a lack of efficacy, hypersensitivity and/ or infusion reactions and/ or
744 development of antibodies directed towards an endogenous available protein resulting in a serious
745 adverse event. If no particular concern or uncertainty arises from the evaluation, inclusion by default
746 of immunogenicity as a potential risk is not required.

747 Since systematic sampling of antibodies might not be feasible in a post-marketing setting, it is
748 important to conclude on potential unwanted immune responses based on suspicious safety and/ or
749 (loss of) efficacy signals, including changes in relevant biomarkers.

750 Within the pharmacovigilance plan of the RMP, the need for additional pharmacovigilance studies
751 should be evaluated and assessed. In case additional studies on immunogenicity are considered
752 necessary the most suitable design should be evaluated based on the aim of the study. At this
753 moment, drug-neutralizing antibodies are not routinely measured in clinical practice. Additional clinical
754 trials or extensions of ongoing clinical trials in the post-marketing setting might therefore be necessary
755 to obtain additional data on the incidence and titres of drug-neutralizing antibodies. Such a trial might
756 also be necessary during biosimilar development in case additional immunogenicity data should be
757 collected in a comparative manner in the post-marketing setting, e.g. immunogenicity data for a
758 chronically administered product has only been collected for 6 months pre-authorisation and additional
759 6 months data is considered necessary by the regulatory authorities.

760 Follow-up of patients treated with a biopharmaceutical during routine clinical practice, e.g. patient
761 registries, has been shown a valuable tool to collect data on the safety of these products. These data
762 sources can also be used for the collection of drug-neutralizing antibodies and adverse events related
763 to immunogenicity, e.g. infusion related reactions. The use of other pharmacoepidemiological data
764 sources should also be explored.

765 Collection of spontaneously reported adverse events should always be done as laid down in the
766 pharmacovigilance legislation. In relation to immunogenicity, spontaneous reporting has been shown

767 an important tool in case of serious safety problems resulting from immunogenicity, e.g. pure red cell
768 aplasia during use of erythropoietins. Other signals related to immunogenicity, e.g. lack of efficacy and
769 hypersensitivity reactions, might also be triggered from spontaneous reporting and should be described
770 in the RMP.

771 The need for additional risk minimisation activities in relation to immunogenicity should be discussed in
772 the RMP and, if considered needed, these activities should be described. Risk minimization activities
773 related to immunogenicity might, among others, consist of guidance in the Summary of Product
774 Characteristics how to measure neutralizing antibodies and deal with the development of neutralizing
775 antibodies.

776 Identification of the product responsible for an adverse event, traceability, is important for
777 biopharmaceuticals. This is especially important for adverse events related to immunogenicity.
778 Traceability is important for both routine pharmacovigilance (collection of spontaneously reported
779 adverse events) and additional pharmacovigilance activities. Appropriate measures to improve
780 traceability, collection of brand name and batch number, should be taken.

781 **10. Summary of the immunogenicity program**

782 Both the planning and the evaluation of immunogenicity studies of a biological product are
783 multidisciplinary exercises. Data that are relevant to the assessment of immunogenicity are dispersed
784 to numerous locations of the marketing authorization application. Therefore, it is recommended that
785 the applicant will include an integrated summary of immunogenicity in the application, including a risk
786 assessment to support the selected immunogenicity program. It is recommended that this summary is
787 placed in chapter 2.7.2.4 Special Studies of the CTD. The summary should be concise and contain links
788 to the appropriate chapters of the application.

789 This summary with risk assessment can evolve through the lifecycle of the product and may be used to
790 support post-authorisation applications.

791 The risk assessment may suggest a low risk. Nevertheless, it is expected that immunogenicity is
792 studied with validated assays according to the scheme in Annex 1. Deviation from this scheme, e.g.
793 omission of the testing for neutralizing ADAs, must be justified. The risk assessment may have an
794 impact on additional characterization of the immune response (e.g. isotyping and epitope mapping),
795 frequency of sampling, timing of the analysis, and selection of the target population.

796 The summary should include at least the following topics when applicable:

797 **Analysis of risk factors**

- 798 1. Previous experience of the product/product class
 - 799 a. does the product have an endogenous counterpart
 - 800 b. do animal models provide useful data of potential consequences of immunogenicity
801 (e.g. elimination of an endogenous protein)
 - 802 c. are there known antigenic sites of the molecule
 - 803 d. attempts to reduce the immunogenicity of the product before and during clinical trials
804
 - 805 2. Physicochemical and structural aspects
 - 806 a. Are there potentially immunogenic structures, e.g. sequences that are foreign to
807 human
 - 808 b. Expression construct and the posttranslational profile e.g. non-human glycosylation
809 patterns/glycans
 - 810 c. Stability and impurities (e.g. presence of aggregates (as visible or sub-visible particles)
 - 811 d. Formulation and packaging, e.g. potential impurities and leachables
-

- 812 3. Does the route and/or the mode of administration raise concerns
813 4. Patient- and disease-related factors
814 a. State of the immunological tolerance
815 i. prone to autoimmune reactions
816 ii. lack of immunological tolerance, e.g. defects in genes coding for endogenous
817 proteins
818 iii. concomitant immunomodulative therapy
819 b. Pre-existing immunity
820 i. "natural" antibodies
821 ii. cross-reactive antibodies, e.g. due to previous therapy with related substances
822

823 **The risk-based immunogenicity program**

- 824 5. Assay strategy
825 a. Rational for the choice of assays
826 i. screening and confirmation
827 ii. neutralizing
828 iii. other, e.g. immunoglobulin class, sub-class
829 b. Specificity and sensitivity of the selected assays in the context of the particular product
830 i. selection of the positive control(s)
831 ii. determination of the threshold for ADA-positivity
832 c. Drug tolerance of the assay at therapeutic concentrations
833
834 6. Approach to immunogenicity in clinical trials
835 a. Sampling for immunogenicity testing
836 b. Justification for the length of the follow up
837 i. on-treatment
838 ii. off-treatment, post-exposure
839 c. Pharmacokinetics
840 d. Pharmacodynamics, efficacy and safety trials
841 i. how the program aims to reveal the incidence, persistence and clinical
842 significance of potential ADAs
843 ii. antigen tolerance of the ADA assay and the trough concentrations
844 iii. loss of efficacy, hypersensitivity, autoimmunity
845 1. definitions and symptom complexes¹
846 2. analysis of clinical correlations of ADAs
847 7. Impact on the risk assessment on the immunogenicity program

848 **Immunogenicity results**

- 849 8. Immunogenicity in clinical trials (relative immunogenicity in case of manufacturing changes
850 and biosimilars)
851 a. (Relative) incidence of ADAs, including neutralising ADAs
852 b. (Relative) titres and persistence over time
853 c. Further characterisation if appropriate, e.g. immunoglobulin classes, cross-reactivity
854 with related therapeutic or endogenous proteins
855 d. Impact of ADAs on pharmacokinetics
856 e. Impact of ADAs on pharmacodynamics, efficacy and safety
857 f. Impact of pre-existing antibodies on pharmacokinetics, safety and efficacy

858 **Conclusions on the risk(s) of immunogenicity**

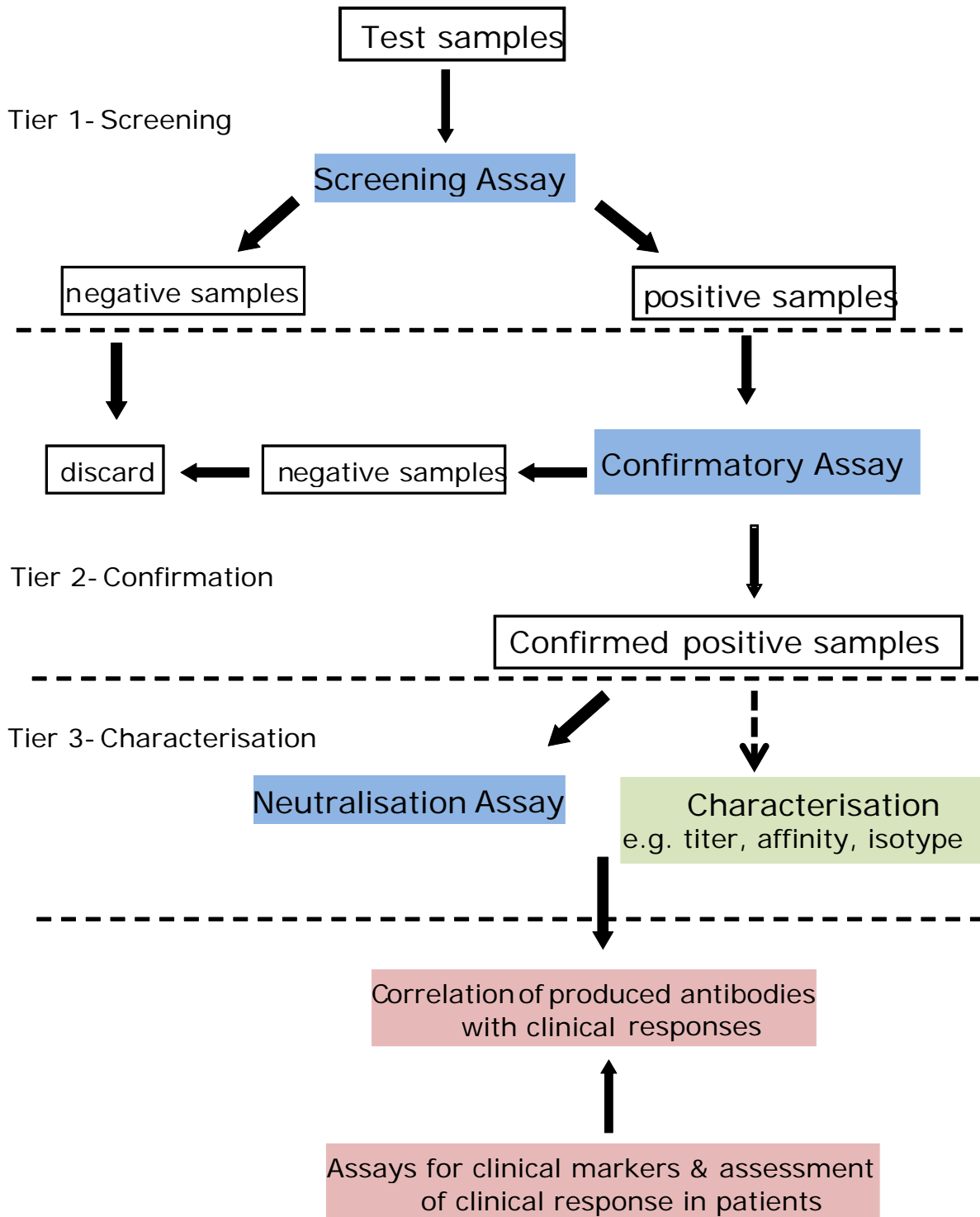
- 859 9. Impact of the immunogenicity on the benefit/risk
860 10. Tools to manage the risk
861 a. Identification of risk groups

- 862 b. Is there a safe level or type of immunogenicity
863 c. Pre-medication
864 d. De-immunisation
865 e. Risk detection and mitigation tools
866 11. How to link adverse events to immunogenicity post-marketing

867 ¹ The Applicant should systematically use terminology and definitions to characterise potentially
868 immune-mediated symptoms according to relevant publications (e.g. Kang P and Saif M. Infusion-
869 Related and Hypersensitivity Reactions of Monoclonal Antibodies Used to Treat Colorectal Cancer—
870 Identification, Prevention, and Management. *Journal of Supportive Oncology*, 5, 451–457)
871
872

873 **Annex 1: An example of a strategy for immunogenicity**
874 **assessment**

875



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877