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# Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use.

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*Disclaimer: This guideline is intended as an addendum to Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins EMEA/CHMP/BMWP/14327/2006 and should be read in conjunction.*


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# Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use.

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# Executive summary

This guideline addresses issues relating to the unwanted immunogenicity of monoclonal antibodies (mAbs) intended for clinical use. These include factors impacting on immunogenicity of mAbs, the clinical consequences of immunogenicity, assay related problems, assessing neutralizing antibodies induced by mAbs and consideration of a risk-based approach for the evaluation of immunogenicity of mAbs.

# 1. Introduction (background)

Immunogenicity can be a significant problem in the treatment of patients with therapeutic biologicals and this is addressed in the 'Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins' by the CHMP (adopted April 2008, referred to henceforth as 'the general guideline'), which in principle is applicable to monoclonal antibodies (mAbs). While many aspects of immunogenicity of mAbs are not different from those for other therapeutic proteins, there are several aspects that require more specific considerations. MAbs are not expected to induce antibodies that cross-react and neutralize an endogenous counterpart (as can occur with EPO) and are not used as replacement therapies. Often mAbs are used as therapeutic or diagnostic agents where alternative treatments or diagnostics may exist. However, some specific aspects of immunogenicity are exclusively or primarily relevant for mAbs or novel mAb derivatives (e.g. Fab fragments, scFv, nanobodies, minibodies) and these are addressed in this guideline.

MAbs comprise a large important class of therapeutic biologicals. The range of clinical indications with potential for treatment with mAbs is very wide. Many mAb products are known to be associated with unwanted immunogenicity and in some cases the immunogenicity causes impaired clinical responses or rare serious adverse reactions which require clinical intervention. The wide range of mAbs in development, and approved for different clinical indications precludes specific guidelines that are pertinent to all situations.

# 2. Scope

The general principles adopted and explained in this document apply to the development and systematic evaluation of an unwanted immune response against a therapeutic or in vivo diagnostic mAb in recipients. The guideline applies to mAbs, their derivatives, and products of which they are components, e.g., conjugates, Fc linked fusion proteins.

This guideline considers the major quality and clinical aspects that are important for adequately addressing the problems with detection of and risk related to the development of an unwanted immune response to the particular mAb in the particular clinical indication sought.

This guideline is aimed at products at final development stage (e.g. marketing authorization application stage). However, many of the principles are relevant to earlier phases of development.

# 3. Legal basis

Directive 2001/83/EC, as amended in particular in Directive 2001/83/EC Art 10(4) and Part II of the Annex I of Directive 2001/83/EC, as amended. This guideline should be read in conjunction with other relevant guidelines, e.g.:

- Guideline on similar biological medicinal products (CHMP/437/04).

- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CPMP/42832/2005).
- Guideline on production and quality control of monoclonal antibodies and related substances (EMEA/CHMP/BWP/157653/2007).
- ICH guideline S 6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA/CHMP/ICH/731268/1998).
- Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins (EMEA/CHMP/BMWP/14327/2006).
- European Pharmacopeia monograph on monoclonal antibodies.
- Guidelines on comparability of biotechnology-derived medicinal products after a change in the manufacturing process: non-clinical and clinical issues (EMEA/CHMP/BMWP/101695/2006).
- Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009).
- Eudralex Volume 9A of the Rules governing Medicinal Products in the European Union (Part I, Chapter 3: Requirements for Risk Management Systems) to be replaced by Good Vigilance Practice (Volume on Risk Management Systems) after July 2012.

# 4. Problems experienced with screening and confirmatory assays used in assessing immunogenicity of mAbs

## *4.1. Assays for antibody detection*

In principle, any immunoassay format can be used to measure antibodies against mAbs. However, assays used to detect antibodies against mAbs are often more problematic, difficult and can be technically challenging. Many standard assay formats involve the use of anti-immunoglobulin reagents such as antibodies against immunoglobulins, protein A or protein G, but these are inappropriate for use in detecting antibodies against mAbs as they very often bind to the product itself. Thus, for example simple ELISAs and radio-immunoprecipitation assays are not usually suitable for use with mAbs unless they are adapted to overcome this problem. Therefore, different assay approaches have to be developed for mAbs. A common approach is to use the 'bridging' format e.g. for ELISAs or electrochemiluminescence (ECL) assays which does not require anti-immunoglobulin reagents and so can be directly applied to studies with mAbs. In some cases, this procedure may be less sensitive than other immunoassay methods and may require significant development effort to produce a suitable assay. It also will not efficiently detect the $IgG_4$ antibodies which can be produced in some cases. Another approach is to use a Surface Plasmon Resonance (SPR) procedure. This does not require anti-immunoglobulin reagents for detecting antibodies against mAbs. It is a real-time procedure and is therefore fast and also detects rapidly dissociating antibodies which can be missed by other methods. However, as SPR simply detects protein binding to the coated chip it needs to be confirmed that the signal is caused by antibodies. It may be less sensitive than other methods for detecting high affinity antibodies and, in the absence of automated sampling systems may have a low throughput.

Samples (normally serum or plasma) may contain substances that interfere with the assays i.e. matrix effects which produce false positive or negative results and/or incorrect assessment of antibody content. Well known examples of such interfering substances are complement components, mannose binding protein, Fc receptors, soluble target molecules, complement receptor 1 and rheumatoid factors, but other substances including the product itself can also cause problems. Assays often need to be 'tailored' to reduce artefacts and achieve acceptable background signal levels, sensitivity and specificity. Applicants need to justify the suitability of the chosen approach, taking into full consideration the limitations of the respective methods.

## 4.2. Presence of mAb product in samples for analysis

Intact mAb products have relatively long half lives and persist in circulation for long periods. Even fragments can persist in blood for several days. This can cause significant problems in detection of antibody responses due to the presence of mAb product in samples collected for antibody assessment. This normally results in an artefactually low estimate of antibody content of affected samples and can be so pronounced as to cause false negative results. Several approaches have been proposed to overcome this problem. One possibility is to delay sampling until levels of mAb product have declined sufficiently to no longer cause problems. This has been claimed to resolve the problem with some mAb products, but requires careful assessment as it has the potential to fail to detect immunogenicity, as induced antibodies may have declined to undetectable levels by the time the samples are taken. Another approach is to use methodology which is least affected by the problem. Some ECL based immunoassays seem much less affected by residual product in samples than other methods, including conventional bridging ELISAs. A commonly described procedure for dealing with the problem is to include a preliminary antigen-antibody dissociation step in the assay design so that any complexes present are disrupted before antibody is detected. Various versions of assays which include acid incubations, sometimes coupled with affinity separation of product have been described for this but need to be carefully evaluated to show that the additional steps do not invalidate the assay. A final possibility is to dilute samples so that residual product present is insufficient to interfere with the assay. This approach needs care as it may result in a false negative assessment of immunogenicity if the assay is not sufficiently sensitive to detect antibodies in the diluted samples. In some cases it may be necessary to assay samples for the amount of residual mAb. In many cases, anti-mAb method development, validation and testing utilizes a combination of all three approaches to reduce product interference.

## 4.3. Confirmatory Assays

Confirmatory assays can suffer from the same problems as screening assays. It is important to select an appropriate confirmatory assay taking account of the characteristics of the screening assay. Use of Protein A and Protein G may be appropriate in confirmatory assays to demonstrate that the positive response is due to an immunoglobulin; however other approaches can be used for this.

## 4.4. Controls

Generation of positive control sera is in general a critical issue for immunogenicity studies for mAbs. The chosen positive control serum or purified antibody is important for monitoring assay sensitivity and specificity. If human sera are not available (as is possible during early phases of product development) then use of animal sera is the only option. Choice of species for this has important consequences. Non-human primates produce significant anti-CDR and anti-framework responses against human or humanized mAbs, which may closely mimic human responses and may be an appropriate positive control. However, non-primate species usually produce antibodies primarily against the constant regions of the mAb, which is unlike human responses. Use of an anti-idiotypic antiserum or mAb can, in some cases, provide a useful positive control. Selection of appropriate negative controls is important. For confirmatory assays, spiking samples with an irrelevant mAb can be used to confirm specificity.

# 5. Assessing the neutralising capacity of antibodies induced against mAbs

MAbs exert their action by various mechanisms ranging from simple binding to antigen, which alone mediates the clinical effect, to binding antigen and mediating one or more immunobiological mechanisms which combine to produce the overall clinical response. Therefore, although simple binding may seem to be the only mechanism operating to achieve clinical efficacy, other effects may also play a role in this. In some cases multiple functions of the mAb may be involved in an additive or synergistic manner to produce an overall combined clinical effect and this may be hard to dissect experimentally to allow a clear understanding of how the mAb mediates its clinical potency. Therefore, if intact mAbs are used, care must be taken not to assume that the Fc mediated immunobiological effects of the product are not involved in clinical efficacy, even when simple antigen binding is considered to be the primary mode of action. In this regard, use of a cell based assay for measuring neutralization has an advantage. In such cases a thorough biological characterization of the mAb must be undertaken, using appropriate biological and immunological assays. Following this, the properties of the mAb need to be assessed to allow selection of an appropriate neutralizing assay strategy.

Antibodies which neutralize the biological activity of biological products may diminish clinical efficacy of the product. It is normally expected that the neutralizing capacity of any antibodies induced will be measured. Any deviations from this need to be justified. For most biological products, the most appropriate neutralizing antibody assay is a bioassay which measures the neutralization of the bioactivity of the product by antibodies. However, the nature of the clinical mode of action of mAbs implies that induced antibodies which block mAb binding to target are those which are mostly associated with reduced clinical efficacy. Therefore, competitive ligand binding assays may be the neutralizing assays of choice for mAbs rather than classic bioassays. This distinguishes mAbs from other classes of biologicals with regard to immunogenicity assessment.

# 6. Immunogenicity risk management of mAbs

## *6.1. Risk identification*

The immunogenicity of mAbs is complex and there are a number of often poorly understood factors which makes it difficult to predict with any certainty whether a therapeutic or diagnostic monoclonal antibody is likely to provoke a clinically relevant immune response. In vitro non-clinical approaches aimed at identifying T-cell epitopes have been developed but these have limited capacity to predict immunogenicity of a therapeutic in humans. However, such procedures can be useful for selecting candidate molecules for further development.

Standard aspects of immunogenicity as described in the general guideline should be addressed for every new therapeutic mAb, taking into account its characteristics, the nature of the intended use and the therapeutic indication.

Preliminary immunogenicity data from early clinical studies can provide information which may be of use for planning later studies, e.g. exploring the performance of bioanalytical assays, detection of pre-existing antibodies or other factors that could confound recognition of treatment-emergent antibodies against mAbs. Based on a risk identification and assessment strategy as further described below, the standard immunogenicity testing programme may be reduced with thorough justification, or may need to be intensified, depending on the level of risk identified. The applicant should always present a thorough risk identification that takes full account of the nature of the product along with to its intended use.

a) Prior knowledge

Available knowledge or lack of knowledge concerning other similar mAbs (e.g., from the same target class, expressed in the same expression system) is an important consideration. The risk perception may be higher if the methodology to either detect antibodies against mAbs or to detect clinical consequences (e.g. mAb trough concentration, PD parameters and response to mAb treatment) of antibodies against mAbs is not sufficiently sensitive. In such cases, more extensive monitoring of the anti-mAb response dynamics relative to therapeutic outcome may have to be considered.

b) Mab structure

In principle, antibodies can be produced against various epitopes present on different parts of the mAb molecule e.g. variable or constant regions. For heterologous e.g. rodent sequence or chimaeric mAbs, recognition of the antibody as being foreign is the primary basis for antibody mediated immunity and antibodies can be elicited against any part of the molecule. With humanised or human sequence mAbs the immune response is predominantly anti-idiotypic (as the complementarity determining regions are hypervariable in sequence ), which clearly can compromise clinical responses to the mAb. However, in some cases, antibodies can be induced against the constant region of human or humanised mAbs and this can affect the effector functions of the mAb with potential consequences on the clinical response. There is less clinical experience with emerging mAb based constructs and this may add to the perception of risk. Special consideration should be given to next generation products, for example, bispecific mAbs or mAb fragments and their capacity to expose hidden antigenic determinants.

Altered glycosylation patterns may decrease or increase the immunogenic properties of the molecule (e.g. change in shielding of the protein backbone). Non-typical glycosylation patterns, e.g. as encountered when adopting entirely novel expression systems, may introduce a higher immunogenicity risk as compared to more commonly used expression systems.

Other factors that influence immunogenicity include impurities arising from the production process and other quality attributes. Therefore, the analytical and clinical approach to assess, characterize and potentially mitigate such potential risks may have to be more extensive, and the risk related to the product quality should be appropriately identified.

For example, a mAb against a target where substantial previous experience exists, but that is produced using a novel expression system, may have a perceived low risk as regards its mechanism of action, but a higher risk as regards potential impact of impurities where little experience exists regarding their effects on safety.

c) Mechanism of action:

The mode of action of the mAb (e.g., cytolytic, apoptotic), and especially the nature of the target molecule (e.g., immunosilencing, immunostimulating), needs to be adequately characterized and comprehensively investigated. Antibody responses against mAbs that target the idiotype of a mAb usually result in diminished efficacy. Likewise, the impact of antibodies against mAbs that recognize allotypic or other regions should be considered carefully since immune complex formation may result in undesirable effects in recipients.

Indirect effects of the antibodies induced by mAbs may also be important, e.g. it is possible that mAbs that target molecules involved in signalling cascades may induce antibodies which cross-link the target molecules in an agonistic manner, potentially leading to enhanced activation of the immune system and possibly cytokine release syndromes. This may be difficult to predict at the individual patient level. For agonistic mAbs or for mAbs where cross-linking could on theoretical considerations lead to immunoactivation, applicants should consider careful observation of patients in early clinical trials to see if such events occur.

d) Clinical factors

Immunogenicity is significantly influenced by clinical factors. Immunogenicity for mAbs can be age related e.g. protein turnover is different in children compared to adults and this can result in differences in observed immunogenicity, e.g. for antibodies used in treatment of juvenile arthritis compared to rheumatoid arthritis at comparable doses. Previous exposure to similar or related monoclonal antibodies can also influence immunogenicity. Therapeutic antibodies used with intermittent dosing schemes have a higher likelihood of inducing immunogenicity than when used in a scheduled and repeated dosing scheme.

Whether antibodies against a mAb have clinically significant effects depends on the binding site of the antibody, the affinity of the antibody for the mAb and the titre of the antibodies that develop. Antibodies against mAbs can occur transiently and then disappear during treatment or alternatively persist throughout treatment or for longer. For some mAb therapies, the development of antibodies has no apparent adverse clinical consequences but for others it reduces efficacy or is associated with therapy related adverse events.

## *6.2. Risk assessment*

Numerous factors contribute to the generation of an immune response against a mAb and these need consideration as part of risk assessment.

Factors impacting the incidence and severity of an immune response against a mAb (product-, process- and patient-/disease-related risk factors), can form the basis of an approach where such risk factors are weighed against availability and feasibility of risk assessment (or identification) and mitigation strategies.

Risk identification, based on the factors discussed above, leads to an assessment that integrates the individual risks in clinical context and an appropriately designed immunogenicity programme as part of clinical development. The assessment of risk requires a multidisciplinary approach taking into account all risks identified (e.g. related to product quality control strategy, including product formulation, justification of acceptance limits for product-related variants and process-related impurities). This also implies that the overall risk assessment should be linked to any comparability exercise potentially performed during development, in case changes to the mAb used occur at different stages of product development.

A pivotal aspect of risk assessment is, therefore, the evaluation of the rate of occurrence and the clinical consequences of an unwanted immune response, and if these consequences can be prevented, appropriately measured, and/or treated medically. Depending on the risk identified and available measures to monitor and mitigate that risk, the testing programme for immunogenicity may be less or more than that described in the general guideline. In any case, applicants should justify and discuss their approach.

Depending on the class and subclass of the mAb (which affects immunobiological functions e.g. binding to Fc receptors) or the mechanism of action, individual mAb products may not all have the same clinical consequences associated with an unwanted immune response. For example, mAbs can be neutralized by antibodies resulting in a reduced efficacy, or result in adverse events such as infusion reactions and/or immune complex formation. Such infusion reactions can be severe, but can (unlike allergic hypersensitivity reactions) be potentially handled by appropriate clinical measures such as the use of pre-medication. Likewise, in case of loss of efficacy, the availability of other mAbs or related therapeutic proteins as alternative treatment options may be an important factor for a risk mitigation strategy. As a general guiding principle, sufficient data to estimate the severity, rate of occurrence and detectability of the risks should be presented at the time of applying for a marketing authorisation.

These risks may have to be further substantiated by post-marketing surveillance and monitoring as required.

As a starting point, the following considerations may be helpful for risk assessment and mitigation:

- A risk stratification based on risk identification principles as discussed in the previous section, integrated with product-related factors like for example identification of intrinsic immunogenic motifs, physico-chemical profile including aggregates or other product-related or process-related variants, information from formulation development for example solubility at physiologic pH, location of the target antigen etc.
- Assay performance details as discussed elsewhere in this guideline, especially the extent to which the sensitivity of the selected mAb antibody assay format is compromised by residual circulating product.
- In case of unavoidable assay shortcomings, the availability of measures that can complement mAb antibody monitoring, e.g. PD measurements or PK parameters.
- The availability of assays that detect early immune responses (e.g. early measurement of binding mAbs, measurement of IgM to detect early immune responses).
- The vulnerability of the patient population; therapeutic index; auto-immune status, use of immuno-suppressant co-medication etc.
- In the oncology setting, loss of response may be more difficult to detect than in other clinical conditions, since it may be difficult to relate tumour progression to the development of antibodies; progression of the disease and consequential loss of response to therapy is usually observed in virtually all patients after a certain time, and it may be challenging to distinguish this from effects mediated by antibodies. Therefore, more intensive measurement may have to take place during the clinical trials to estimate what to expect in a post-authorisation setting, especially when therapeutic alternatives are available.
- For administration of the mAb at home or in the clinic: Treatment with mAbs in the clinic may offer advantages for immediate mitigation of infusion reactions or anaphylaxis should they occur, but home use for subcutaneously administered mAbs may offer advantages for patient care. Thus, applicants will have to weigh the risk of unwanted immune responses and their consequences against the intended clinical use. For example, mAbs with a high incidence of reactions following subcutaneous use may be less suitable for home use.
- Availability of alternative treatments or diagnostic procedures in case of loss of efficacy or induction of infusion reactions or anaphylaxis.

## *6.3. Risk monitoring and mitigation*

Following such an approach of risk identification and assessment, applicants should carefully plan this concept early in product development, and revisit and update it regularly during product development and the entire lifecycle of the mAb product as new data become available. At the beginning of clinical development, applicants may, for example, have to assign a higher risk level to the mAb, although the mechanism of action per se may not necessarily suggest a higher risk, if other factors make this necessary. The risk level may, depending on the results of larger clinical trials, need to be re-considered following the trials. At the time of marketing authorisation application, applicants should thoroughly justify and discuss their overall concept for the design and extent of immunogenicity testing used during their development programme.

For products that are claimed to exhibit a particular advantage as regards immunogenic potential, (e.g. a claim in the Summary of Product Characteristics) appropriate data to support this is usually required.

Depending on the outcome of the risk assessment, in some cases more detailed and additional testing may be necessary during clinical trials. For example, in some instances, IgE testing prior to clinical administration needs to be considered for patients if the mAb contains non-human carbohydrate structures, e.g. Galactose-α-1,3-Galactose, in order to prevent severe anaphylaxis. Another instance where development of IgE testing should be considered is where the incidence of allergic reactions was high on first administration during early clinical development of the product. While measuring levels of IgG subclasses, or other Ig classes like IgA, are normally not a standard requirement for immunogenicity assessment of mAbs, they may become necessary as part of such an approach when certain risks have been identified (e.g. nasal application). However, the determination of neutralizing capacity and of transient versus persistent mAbs by repeated sampling is usually required.

The frequency and timing of sampling and analysis may vary depending on the identified relative risk level. For lower risk mAbs it may be possible to reduce sampling frequency in later stages of development, provided that no adverse events or reduced efficacy has been observed. Nevertheless, banking of samples should be undertaken on a routine basis over the whole development programme. Such an approach needs to be duly justified. For mAbs where a higher risk is determined, sampling may have to be more frequent during the whole clinical development. In this situation it may be advisable to analyze samples in real time. It may be necessary during the clinical development to measure antibody levels, PK, PD markers, efficacy and safety simultaneously and over a period of repeated treatments. This allows assessment of the clinical significance of antibody development, and also whether the antibody effect changes over time, which could occur as a result of increase in titre and/or isotype switching/affinity maturation of the antibody response. Non-neutralizing anti-mAb antibodies may indirectly impact efficacy by binding to the mAb product and impacting on its pharmacokinetic properties. Therefore, measurement of PK parameters may be a potential help when planning how to measure an anti-mAb antibody response.

Information derived from assays can be used for risk management. For example, where risk identification and assessment has concluded that early identification of an immune response is required and allows for the possibility of discontinuing treatment with the mAb, development of low affinity IgM antibodies can be an indicator of an early immune response, and measuring IgM responses could help in early identification of patients mounting an immune response. Likewise, the detection of binding, non-neutralizing antibodies may be an early indication of later development of neutralizing antibodies.

Risk mitigation strategies could, for example, include studying how to effectively handle patients that show an immune response, e.g. if dosing schedules can be intensified without compromising patient safety etc. However, feasibility considerations will have to be taken into account as part of this.

When filing a Marketing Authorisation Application, it is recommended that applicants present an integrated summary of their strategy on risk identification, characterisation, monitoring, minimization and mitigation. This risk-based approach should also have been taken into account for the Risk Management Plan (RMP), discussing how risks identified by data from the development programme, and potential risks, or missing information should be handled in a post-authorisation setting.