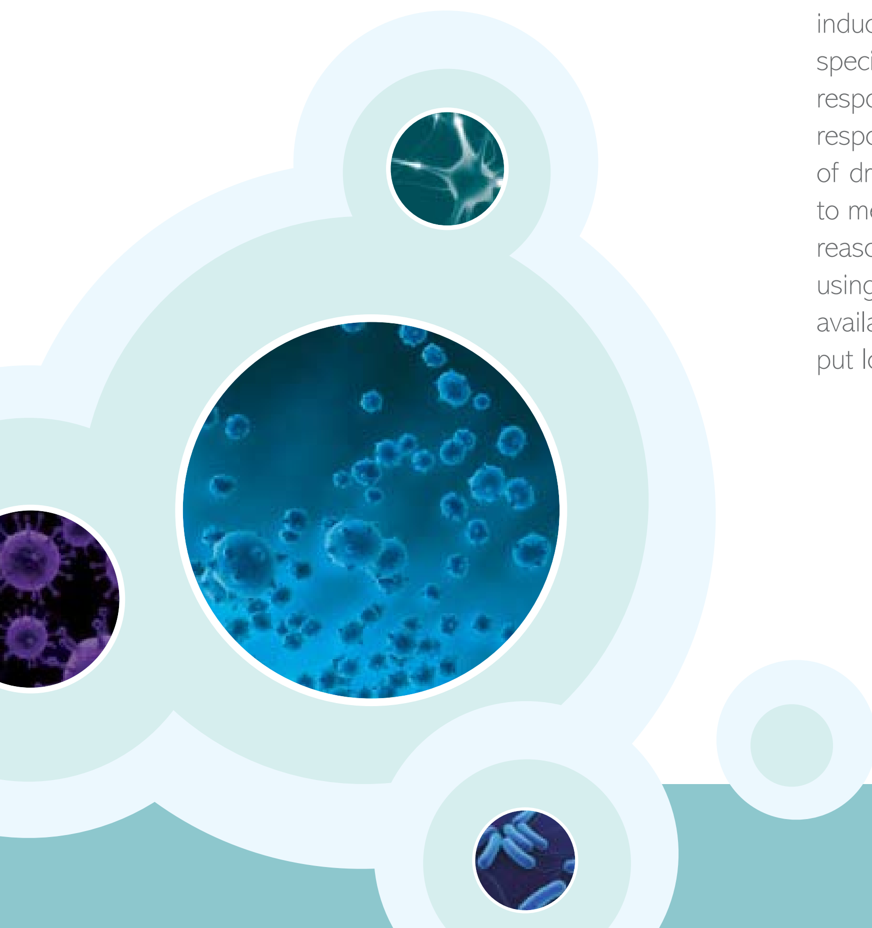
bio outsource
cGMP compliant biotesting

Technical Bulletin: Successful Strategies to Measure Anti Drug Antibodies.

The problem of drug induced immunogenicity has been a recognised phenomenon for a number of years. Anti-Drug Antibody (ADA) responses can lead to allergic reactions, reduction or neutralisation of drug activity and potential cross reactivity. Furthermore, in preclinical studies, ADA can affect drug exposure, in turn affecting the interpretation of the toxicity, pharmacokinetic and pharmacodynamic data. The problems are difficult to predict as immunogenicity is dependent on a number of known and unknown factors. However, it is now clear that as part of a successful clinical trials programme for market authorisation, an assessment of the induction of an immune response by the drug is required. The European regulators have issued a document EMEA/CHMP/BMWP/14327/2006 which came into effect in April 2008 to give guidance on the methodologies expected to be applied to new biotechnology products.

One of the first drugs which was shown to have serious clinical effects from drug induced antibodies was erythropoietin. The clinical use of this drug, involving multiple doses over a relatively short period of time, has a clear effect on the induction of immunogenicity. However what also became clear was that there were many other factors which induced immunogenicity with this product. As erythropoietin approaches the end of its patent lifespan there are a number of other manufacturers who are seeking to produce "Biosimilar" drugs; the question of immunogenicity of these products needs to be addressed.



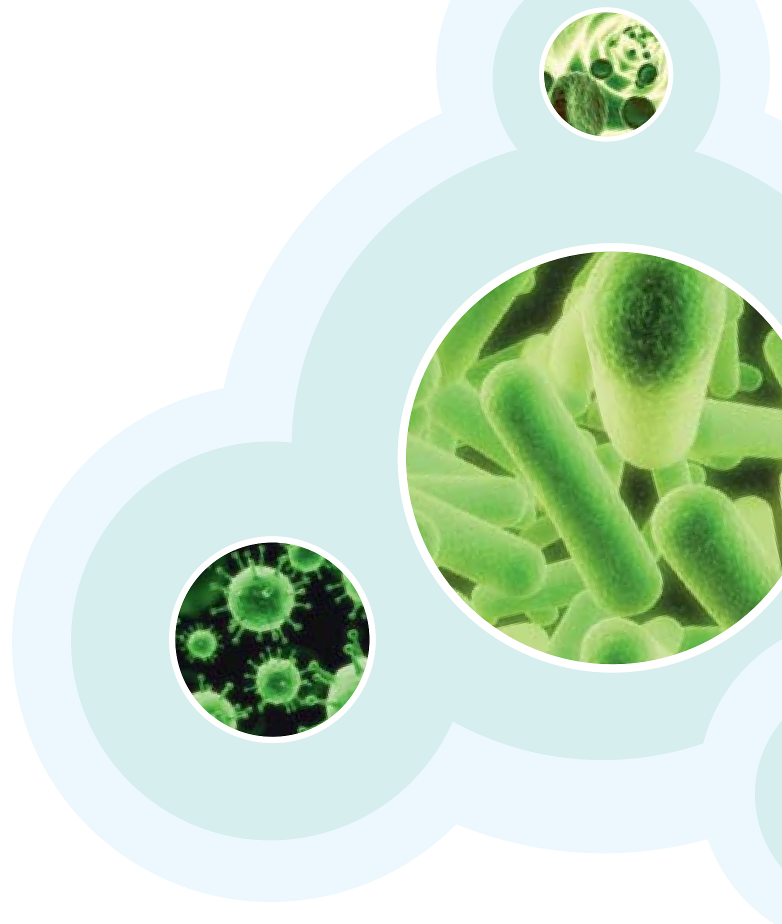
Immunogenicity, when discussed with respect to drug induced immunogenicity, normally refers to a drug specific antibody induced immune response. Antibody response is sometimes only a small part of the immune response to any antigen; however the measurement of drug induced immunogenicity is typically limited to measuring the antibody response for a number of reasons. Measuring ADA is a well established process using ELISA technology as its basis. Technology is available in an easy to use format, enabling high through-put low cost testing of clinical samples for ADA.

ELISA techniques to detect drug antibodies present in a serum or plasma sample typically use a direct mode with the target compound bound to an ELISA plate. The detection of the bound antibody is evaluated using a secondary detector antibody, to the human immunoglobulin, which is tagged with an enzyme. The enzyme catalyses a chemical reaction which results in a measured colour change or in some cases the induction of light [luminescence]. The drug capture can be accomplished by a number of means and is one of the critical steps in the assay. The sensitivity of ELISA techniques is well recognised to be very good and, in our hands, detects low µg quantities of antibody in a sample. The limitation in sensitivity of ELISA techniques is the detection of signal to background measurement. Some newer technologies, such as MSD™ (Meso Scale Discovery), use the basic ELSIA format but employ electro-chemiluminescence methods to measure bound antibody which can increase sensitivity by up to 3 logs over traditional techniques.

The difficulties in setting up an ELISA technique are not based on the technology but rather due to the limitations of controls which can be used to qualify and validate the assay. Frequently we are limited in the number and volumes of human anti-drug serum or plasma samples we have available. In many instances there are no human samples available and samples collected from animal which have received multiple doses of the drug. This is unavoidable during the pre clinical or phase 1 studies since there are no human samples available. However, when such samples become available they should be used to qualify the assay. When using animal samples care should be taken to use animals which are dosed with similar material to that which is used in the human studies. It is also useful to analyse samples harvested from as many different animals as possible which have been dosed with the drug under analysis. This gives information on the spectrum of response from different animals and may indicate the potential responses seen in human subjects. Other frequently used serum controls include those harvested from rodent, goat or sheep serum. Primate samples, which may be more indicative of likely human immunogenicity, are less available. These samples can be limited in volume, difficult to come by and should be retained as part of any pre-clinical studies rather than an afterthought as is the case in many instances. Caution should be taken with some samples taken from animal studies as the animals are frequently dosed with very high amounts of drug relative to body weight and in some instances an animal's pharmacokinetics are very different to humans. These may result in instances where there is a level of drug product still in the sample and this may mask the presence of antibody. These assays should be validated to the ICH guidelines for reproducibility, accuracy, linearity, limit of detection etc.

The ultimate result from ELISA assays is a measure of the total antibody present in a sample which is reactive with the drug under test. While this is valuable data, it does not indicate the ability of this antibody to interfere with the activity of the drug *in vivo*, nor does this measure the ability of the drug to react with any native proteins. These questions are of critical importance in estimating the ADA related risk of these drugs and also in the management of patients following induction of an immune response. In the second stage of analysis, ELISA technology is replaced with an assay to determine the biological neutralisation ability of the antibodies against the drug activity. For this assay a biological measure of the drug activity is required. The biological measure usually takes the form of a cell based assay which measures the effect of a drug on specific cell types [in some instances it is only possible to measure this activity in an animal model. In such cases the animal model must be used]. The neutralisation assay allows the drug to react with a sample of serum or plasma under test. After an incubation period the sample is then added to a cell based assay to detect any reduction in activity of the drug. If a potency assay is not available then this must be developed prior to investigating the immunogenicity. In some instances monoclonal antibodies can be created against epitopes thought to neutralise drugs and these can be used to establish a neutralisation assay. However, caution should be used when interpreting these results as the highly concentrated monotypic response achieved by these reagent can be very different to that produced by the polyclonal in the typical immunogenic response.

bio outsource
cGMP compliant biotesting



BioOutsource firmly believes that assay validation is an ongoing process and validation should focus on making assays fit for their intended purpose. Validation studies will be performed to establish that the assays show appropriate linearity accuracy, precision, sensitivity, specificity and robustness. Assays are also validated to show the matrix effects caused by reagents or substances present in samples and controls and steps are always put in place to minimise these. Drug product interference is also established for each assay and matrix. In some instances it may be required to investigate drug immune complexes by acid dissociation; this can be carried out as an investigational study for our clients as samples require.

BioOutsource can offer:

Screening assays

BioOutsource can offer the development of product specific ELISA based screening assays capable of detecting antibodies induced against the biological product in animal and patients samples. We can source the relevant negative and positive control serum (human or animal) and qualify these as necessary. BioOutsource will validate these assays to ICH guidelines including sensitivity, specificity, precision, reproducibility and robustness. Other non-ELISA technologies are available dependant on the requirements of the samples.

Confirming the presence of antibodies

These assays are necessary for elimination of false positive samples/patients following the initial screen. Typically these are based on similar methodologies to the screening assays but will use different targets or other methods to establish and confirm the presence of reactive antibodies.

Neutralization assays

BioOutsource can transfer or develop a biological assay to detect the presence of neutralising antibodies in patients or animal serum samples. Typically these studies require a number of months of development and preparatory work prior to the screening of patient's samples. Neutralisation assays can be adapted from the biopotency release tests applied to the product. These assays must be validated in a similar manner to the screening assays, again following the ICH guidelines.

New technologies

The Mesoscale technology (www.meso-scale.com) has the potential to revolutionise our abilities to measure ADA. The technique will allow the measure of low affinity and low avidity antibodies which may be crucial in understanding the extent of ADA. This technology has been used for a number of years and has already been shown to surpass the standard ELISA technology in both sensitivity and specificity and as such it is likely to become the method of choice for these studies. For more information contact info@biooutsource.com.

BioOutsource Ltd • Units 3/4 Technology Terrace • Todd Campus • West of Scotland Science Park • Glasgow • G20 0XA
tel: +44 (0)141 946 4222 • email: info@biooutsource.com • web: www.biooutsource.com