

Immunogenicity Assessment of Therapeutic Proteins and Peptides

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Abstract: Assessment of immunogenicity is a major aspect in evaluating the safety of biological therapeutic proteins. It is important to evaluate the immunogenic potential of the biologics in an appropriate fashion using clearly defined strategy and clinical trials. The studies must include the appropriate risk assessment procedures using validated methods. The immune responses against the therapeutic biologics can be studied using various methodologies. These include enzyme linked immunoassays (ELISA), surface plasmon resonance (SPR), chemiluminescence, and flowcytometry assays for binding antibodies and cell based assays for neutralizing antibodies. The immune responses to the biologics can widely vary in various cross section of the population, thus a combination of techniques are necessary to fully evaluate the immunogenic potential of the biologics. This review outlines various commonly used technology platforms, its merits and shortcomings for the evaluation of the immune responses.

1. INTRODUCTION

Immune response or immunogenicity of the therapeutic proteins and peptides remains one of the major risks. The testing of the immune response to these biological products such as the anti-drug antibodies (ADA) are an important part of the product development and post marketing monitoring of drug safety [1]. The major causes of immunogenicity to the biological products are still unclear, but factors such as aggregation are suspected to trigger an immune response [2-4]. The key challenges is that proteins can degrade both by chemical and physical degradation pathways. This protein physical degradation results in the generation of protein aggregates. The ability of proteins to aggregate has been recognized from the early beginnings in protein biochemistry [5]. Physical degradation of the protein can occur during the production and purification but can also result from improper formulation, storage or handling conditions [6]. Aggregates have shown to increase the immunogenicity of various therapeutic proteins, which might be explained by their multiple-epitope character and/or to conformational changes of the individual aggregated protein molecules [7]. Insulin aggregates has been shown to induce antibodies against insulin. There is evidence that the aggregates possess the antigenic epitopes [8]. The presence of protein aggregates in formulations of human growth hormone has been correlated with an increased frequency of immune responses. The anti-drug antibodies can affect or alter the pharmacokinetic (PK), pharmacodynamic (PD), bioavailability and efficacy of the drug [9]. The antibodies against the drug might trigger a range of safety issues from mild to adverse side effects. In the most severe form it can induce autoimmune disease like conditions, such as thrombocytopenia [11] and pure red blood cell aplasia (PRCA) [12] by neutralizing the endogenously secreted proteins. Thus it is essential to monitor the immunogenicity of the therapeutic proteins and characterize

the antibodies in terms of binding and neutralizing ability using appropriately validated assays [13-15].

A risk based approach strategy has been proposed for the antibody testing [16, 17]. This approach is based on the level of harm or adversity that an antibody to the therapeutic protein will elicit to the patient. When the risk is greater, the assays must be able to detect the formation of an antibody to the therapeutic protein and/or its native endogenous protein. An endogenous enzyme or a growth factor replacement therapy will be at high risk compared to a monoclonal antibody therapy. The anti-therapeutic protein antibody not only will affect the efficacy of the therapeutic, it might also hinder the native endogenous activity of the protein. The selection of the assay format directly influences the anti-drug antibody testing and its outcome. Various methods used have their own advantages and disadvantages. Each of these methods has limitations that might hinder their use in a particular situation. Various formats of the assays that are commonly used for the assessment of the immunogenicity for both binding and neutralizing antibody are discussed in this chapter.

2. ANTIBODY TESTING STRATEGY

The anti-drug antibody testing involves a two tier approach [17, 18]. Initially the sample is tested for the presence of the anti-drug antibodies using a binding assay followed by the ability of those antibodies to specifically neutralize the drug. Additional characterization of the immune response in terms of isotype of the antibody as well as affinities has also been reported [17]. A representative antibody testing strategy is given in Fig. (1). Samples for antibody testing are initially analyzed using a screening immunoassay. Samples that yield a signal below the immunoassay's cut point for antibody detection are negative. Samples that are signal equivalent to or higher than the immunoassay's cut point are tested in a confirmatory immunoassay. The samples that are positive are further tested in a cell-based or competitive ligand binding assay for presence of neutralizing antibodies. Under certain circumstances other characterization immunoassay test-

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ing may also be performed on samples that test positive in the confirmatory immunoassay. These tests may include determination of the isotype, IgG sub-class, dissociation rates, affinity and relative concentration of the antibodies.

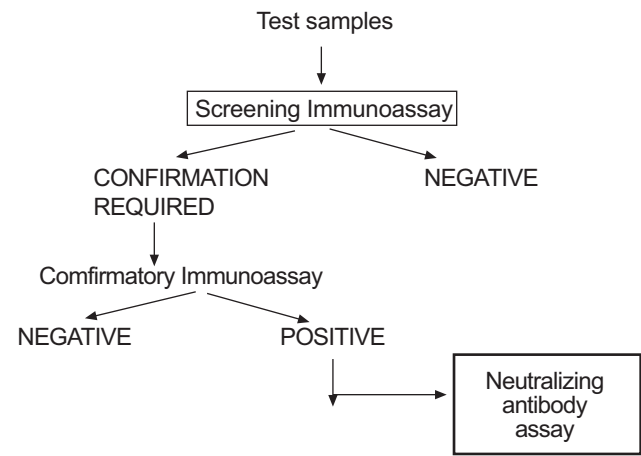


Fig. (1). Anti-drug antibody sample testing strategy.

3. RISK BASED ANTIBODY TESTING

The antibody testing should consider the severity of the consequences of an antibody response to the therapeutic protein. Factor such as biological function or cross reactivity to endogenous proteins and health status should be taken into consideration while making the decisions on the assay format. The chosen format should detect the clinically relevant antibodies. An example of high risk will be an endogenous version of the protein used as replacement therapy and used repetitively in life threatening situation. A non-endogenous, redundant or non-replacement therapy to be used as single dose treatment will fall under a lower risk category.

4. BINDING ANTIBODY TESTING

The binding antibody testing commonly uses three different platforms. Binding immunoassays such as direct binding, sandwich and bridging formats are extensively used [19-22]. The second platform is based on the mass related immunoassay using mass spectrometry or surface plasmon resonance (SPR) [15, 18]. The mass based technique has been reported to detect more of the lower affinity antibodies to the drug. The third and recent technology is bead-based cytometry detection of antibodies in serum using flow cytometry [19, 23]. In all the assay platforms it is very essential to choose the format that detects the anti-drug antibody with appropriate sensitivity. Table 1 describe the various forms of the assays used for the detection of biding anti-drug antibodies. The details of the assays are described below.

4.1. Sandwich Immunoassay

In sandwich immunoassay the drug is coated or captured on to a plate directly, by passive adsorption or using biotin/avidin tagged drug (Table 1). This is followed by the exposure to the test sample serum that contains the anti-drug antibody. The bound antibody is revealed by using a secondary antibody labeled with either enzyme or Chemilumines-

cence responsive tag (e.g. ruthenium). A variation to the sandwich immunoassay, where the drug is coated in limiting quantity followed by the test serum addition, is the bridge format (Table 1, second row). In this format, a plate is coated with the drug and is exposed to the test serum. The difference from the other sandwich assays is that the labeled drug is used to form a bridge. Thus this requires that one of the sites in the antibody be available for binding to the labeled drug. This assay format and technique is most versatile and has been very widely used for the detection of anti-drug antibodies. The ease of use and the reliable cost effective read-out technology give optimal sensitivity and quick turn around time. The limitation of this technique is the repeated washing steps necessary after addition of each of the reagents that reduces the detectability of relevant low affinity anti-drug antibodies. Also, in certain disease population, serum results in higher background due to non-specific binding.

Table 1. Assay Formats Commonly Used in Binding Anti-body Assays

Technique	Assay Format
Sandwich, (ELISA, Chemiluminescence)	
Bridging (ELISA, Chemiluminescence)	
Mass related	
Cytometry based	

Detection agent (Enzyme or Chemiluminescence Tag)

Anti-drug antibody

Labeled Secondary detection antibody

4.2. Mass Related Immunoassay

In mass related immunoassay, the binding of antibody to the drug remains similar to the other immunoassays. The detection is based on the change in mass due to the formation

of the immune complex, and is detected either by mass spectrometry or using SPR. The drug is coated onto the surface either directly or captured using the avidin/biotin chemistry (Table 1, third row). The immune complex formed by interaction with the serum anti-drug antibody is revealed by the changes in the light characteristics. This technique can be used without any modification or labeling of the drug. In recent reports it has been shown to detect low affinity relevant antibodies [24]. The widely used SPR technique has higher turn around time, but also usually has lower sensitivity; however, this is offset by its ability to detect lower affinity antibodies as well as to get more detailed characterization on the affinity and dissociation rates [18].

4.3. Cytometry Based Immunoassays

This new technique uses the same basic principals on the anti-drug antibody interactions with the drug forming the immune complexes. The detection is based on the fluorescence-labeled revealing reagents. The beads are coated with drug or captured on to the beads using the avidin/biotin chemistry followed by the secondary revealing fluorescence labeled antibodies or in a bridging format with the labeled drug [19]. Multiplexing of the assay can be easily done using this technique to detect cross reactivity of the antibodies with other related molecules [23, 25]. The limitation of this technique is similar to the sandwich and bridging immunoassays, i.e., need for conjugation of the reagents as well as repeated washing of the beads.

4.4. Acid Dissociation Based Assay for Drug Interference

All assays mentioned above measure the anti-drug antibodies in serum from the subjects who were exposed to the drug. If the sample had free drug from the prior exposure, they might interfere with the detection of anti-drug antibodies. Various techniques to reduce the effect of free drug have been used. The removal of the free does not increase the sensitivity of the assay and also might remove the complexes of drug/anti-drug antibodies. The acid dissociation technique [26-28] gives the ability to detect the anti-drug antibodies by dissociating the immune complexes. Fig. (2) gives a schematic view of the technique. The serum sample containing the anti-drug antibodies and the drug immune complexes are dissociated by subjecting them to lower pH (pH 3.0). The samples are then incubated for a specified amount of time, usually 15 min to 2 hr. Then the pH is adjusted to neutral (pH 7.0) while adding 10 - 100 molar excess of the labeled drug and incubated on to a plate coated with the drug. The bridged anti-drug antibody is revealed using the bound labeled drug. This acid dissociation technique greatly improves detection of the anti-drug antibodies in presence of as much as 50 molar excess of the drug over the antibody without affecting the sensitivity of the assay. The limitations are that all anti-drug antibodies may not be detectable by this technique; especially the very high affinity antibodies may not be dissociated at the pH levels that are safe to treat the serum samples. Moreover the assay involves washing steps that might affect the detection of low affinity antibodies.

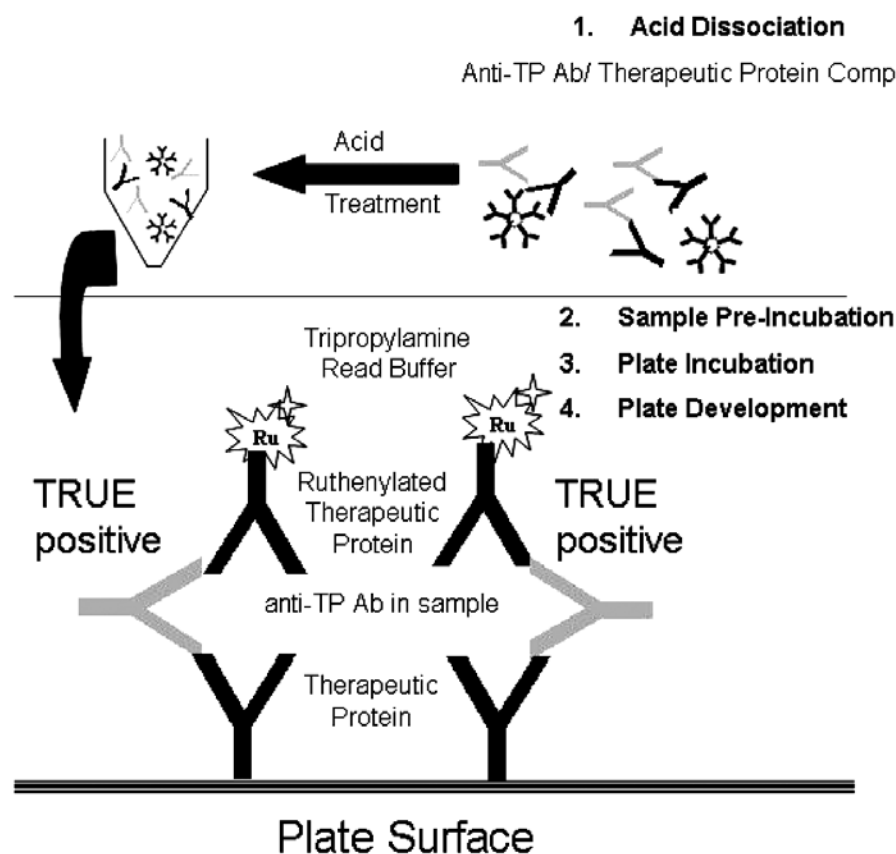


Fig. (2). Acid dissociation bridging assay.

4.5. Considerations for the Binding Antibody Immunoassays

All the assays that depend upon coating to the plates or a solid surface have the potential of masking the epitopes. The conjugation techniques used in the sandwich and the bridging formats can also mask or modify the epitopes due to chemical conjugation at a specific site. Moreover the isotype-specific secondary antibody reagents used to reveal the drug specific-antibody in the direct and sandwich type assays bind to different class to varying extent, resulting in a wide range of signals. To minimize the variation of this signal, a standardized mixture of these reagents can be made and used throughout the study. The bridging format relies on at least one site being available for the labeled drug to bind and make the bridge between the plate and the labeled drug. The bridging can be mediated through non-antibody mediated agents in this assay. This lack of antibody specificity issue could be solved by the use of protein G/L that binds and depletes the antibodies in the test serum.

The sensitivity and affinity play an important role in all these assays. The assays used in the clinical testing must detect the lowest possible levels of the antibody. Use of well-qualified reagents and combination of the various formats provide the more complete analysis of anti-drug antibodies. For example mass related immunoassay such as SPR in combination with a bridging immunoassay will provide the ability to detect the lower and higher affinity anti-drug antibodies. The bridging assay format will provide a better sensitivity for higher affinity antibodies whereas the lower affinity antibodies are best detected by the SPR.

5. NEUTRALIZING ANTI-DRUG ANTIBODY TESTING

The neutralizing antibodies assays are assayed once a sample has been found to contain anti-drug antibodies. Two major formats, cell based and non-cell based receptor or ligand binding/inhibition assays, are used in the detection of the neutralizing antibodies.

5.1. Cell-Based Neutralizing Antibody Assay

A cell-based assay gives the most appropriate model for the detection of neutralizing antibody. The detection is based on the ability of the anti-drug antibodies to eliminate or reduce the biological activity of the drug.

5.2. Cell Line Selection

The foremost importance of the cell line selected in the assay should be representative and be as close to the mechanism of action of the drug. Secondly the cell line should also be amicable to the presence of serum and work over a wide range of serum concentrations. If an appropriate cell line is not available, cell lines can be engineered with appropriate receptors to read the responses to the drug. The cell lines should be well characterized in terms of responses to drug, serum concentration and culture for a period of time before used in the assay.

5.3. Assay Endpoints for Cell-Based Assays

The various commonly used end points of the cell-based assay are proliferation, cytokine secretion, phosphorylation and specific gene expression analysis [29-32, 33]. The assay end points can be an early or late biological response to the drug or its inhibition of the ligand that interacts with the cell. The examples of early time point responses are binding of the drug to the cell surface receptor, its internalization, or early signaling events such as phosphorylation. Table 2 provides most commonly used endpoints. The drug induced cell growth can be monitored by cell proliferation assessment. This can be measured by radioactively labeled thymidine incorporation into the DNA of the growing cells. Alternately, the intra-cellular level of cyclic Adenosine Mono Phosphate (cAMP) (Table 2, first row) can be used as a readout for the cell viability. Protein expression resulting from the drug or ligand action on the cells can be measured as readout, e.g., cytokine induction (Table 2, second row). When the expression of a protein or other signals is difficult to measure due to very low expression, the messenger RNA expression can be measured using a polymerase chain reaction (PCR) or branched DNA technology (Table 2, third row). This technique amplifies the signals by a large degree and yields a very sensitive assay. The response to the ligand or the drug to its receptor can be measured using signaling events, e.g., phosphorylation of proteins (Table 2, fourth row). These responses are relatively fast and can be measured much quicker than other cellular responses, usually in less than 30 minutes. The relatively small window of exposure of the test serum to the cell reduces the deleterious effects of serum on the cells. When there are no specific receptor expressing cell lines available, the appropriate receptors can be engineered along with a reporter (e.g. luciferase) gene (Table 2, fifth

Table 2. Assay Readout Commonly Used in Cell Based Neutralizing Antibody Assays

Assay Readout	Mechanism of Action	Endpoints
Thymidine uptake, cAMP measurement	Induces cell growth or cell death (depending on the format)	Proliferation
ELISA, chemiluminescence	Induces or alters protein expression	Protein expression
Branched DNA technology	Affects mRNA expression	mRNA expression
ELISA, chemiluminescence	Phosphorylates or signal results in phosphorylation of proteins	Phosphorylation
Luciferase, Beta-galactosidase	Affects or alters reporter gene expression	Reporter gene expression

row). As these cell lines are engineered, they can be made to very specific needs, e.g., tolerant to test serum.

5.4. Assay Formats

These assays fall into two categories, i.e., direct and indirect assays. Sections 5.5 and 5.6 describe in detail the direct and indirect assays. Schematic representations of these assays are given in Figs. (3 and 4). The assay format depends on the mechanism of action of the drug. The serum's interaction with the cell line also dictates the format of the assay.

5.5. Direct Assay

In a direct assay such as growth factor binding, drug interacting with its receptor on the cell surface results in proliferation of the cells. The anti-drug antibody will bind to the growth factor resulting in the inhibition cell growth [29] (Fig. 3). Typically the direct assay format is used for the

proteins or drugs that directly act on the system such as hormones and cytokines. The cells are exposed to the drug and the effector function such as proliferation or cell death is read by measuring the ^3H -Thymidine uptake or intracellular cAMP [34]. In direct assay format, due to prolonged exposure of the serum to the cell line, there is a possibility of serum's effect on the assay. To monitor these effects, appropriate "controls" in the assay, e.g., exposure of serum without drug to the cell line, should be included. Cells along with drug and the positive control will provide a good system suitability test for the assay. Any unanticipated or harmful effect of the matrix on to the cell can be monitored using these controls to provide a robust assay. Monitoring of the signal in response to the ligand will provide the viability of the cells and the extent of response over the background will provide the consistency of the response as well as the potency of the drug product used in the assay.

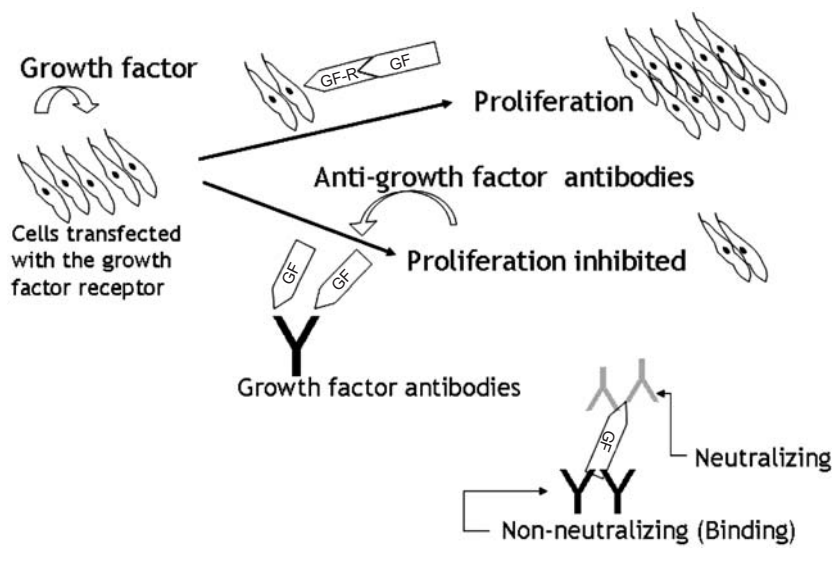


Fig. (3). Direct assay format.

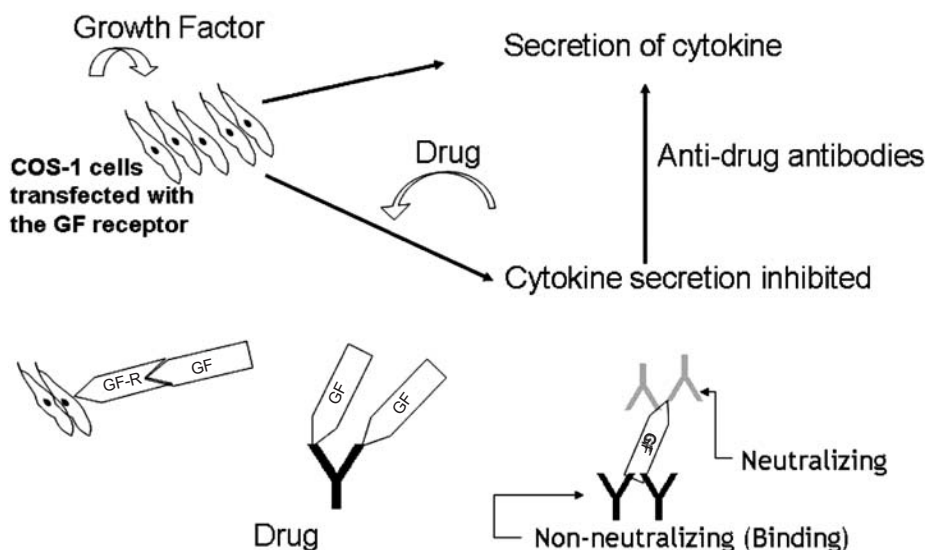


Fig. (4). Indirect assay.

5.6. Indirect Assay

In an indirect assay, typically the drug is an antibody against the growth factor, ligand or its receptors. Products such as monoclonal antibodies, soluble receptor and receptor antagonists fall in this category. Addition of the drug inhibits the ligand-induced response e.g. cytokine secretion; anti-drug antibody will bind and neutralize the drug there by restoring the ligand induced response (Fig. 4). Indirect assays are usually used in monoclonal antibody drugs and soluble receptors. The drug acts on the target of the interest that has the biological effector function. When the drug is added to the ligand, the effector function such as production of cytokine is blocked. The secreted cytokine is measured by an immunoassay. The neutralizing anti-drug antibodies restore the function of the ligand by inactivating the drug. It is necessary to include appropriate controls to monitor the function and potency of those reagents or molecules in each assay.

5.7. Considerations for the Binding Antibody Bioassays

The cell-based assay design should incorporate the following details. The cell line selected should be representative of the mechanism of action of the drug. The format can be decided based on the biological action of the drug on the selected cell line. Once the design is finalized, the assay should be optimized for the correct concentration of the drug to be used in the assay, culture conditions such as cell density, and incubation time, which depend on the assay endpoints for each of the assay.

5.8. Receptor Binding Assay

The receptor binding assay format can be used in cases where the effector cell lines are not available with appropriate specificities and also, if there are issues with the serum, e.g. certain disease serum are not well tolerated by the cells. A schematic representation of the assay is given in Fig. (5). In this example the drug is an antibody against the ligand. Biotinylated receptor is bound to avidin-coated plates. The ruthenylated (RU)-Ligand binds to biotinylated receptor in the absence of drug. Ruthenium is detected by electrochemiluminescence. The drug blocks the binding of ruthenylated (RU)-ligand to biotinylated receptor. Alternately, the receptor for the ligand can be coated on to a plate. The use of avidin/biotin capture chemistry gives the advantage of the assay being done in solution phase. As these assays do not involve the cells, the effect of serum is minimal and higher concentrations of serum can be used in the assay. The non-specific binding can be lowered by additional washing of the plates and the signal can be enhanced using various signal amplification methods.

The limitations include that the receptor for the drug needs to be well characterized and available in a purified form. When multiple isoforms of the receptors are present, the effects on each of them have to be evaluated separately. Even though the receptor protein can bind the ligand, it may not have the same affinity for the cell surface receptor. Chemical modifications of the receptors for the use in the assay might affect its biological activities and their stability has to be evaluated in the assay as well as in storage. The

assay specific controls for the evaluation of these parameters must be included in each of the assays.

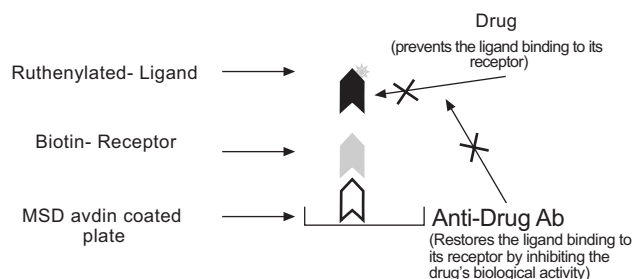


Fig. (5). Receptor binding/Inhibition assay format.

CONCLUSIONS

The immunoassay and the bioassays for the detection of anti-drug antibodies provide a valuable tool and safety evaluation of the therapeutic proteins and peptides. These assays tend to be very complex due the fact that they involve various cell lines and biologically produced therapeutics. The key to achieving robust and good quality assays is to choose the right format, and design and develop them keeping in mind the various parameters involved. The assay design should include the appropriate format, cell line, endpoint, control antibodies and assay controls and also avoid the serum interference. Once the assay is designed, the assay should be optimized for drug product concentration, assay matrix, response range, assay configurations of plates and controls for a reasonable sensitivity. All assays have to be validated for its cut points, sensitivity, precision, robustness, inferences from drug and matrix, stability and ruggedness before use in a clinical evaluation of anti-drug antibodies.

REFERENCES

- [1] Wadhwa, M.; Bird, C.; Dilger, P.; Gaines-Das, R. and Thorpe, R. (2003) *J. Immunol. Methods*, **278**, 1-17.
- [2] Rosenberg, A.S. (2006) *AAPS J.*, **8**, E501-E507.
- [3] Schellekens, H. (2008) *Biotechnol. Annu. Rev.*, **14**, 191-202.
- [4] Kessler, M.; Goldsmith, D. and Schellekens, H. (2006) *Nephrol. Dial. Transplant.*, **21** (Suppl 5), v9-v12.
- [5] Shire, S.J.; Cromwell, M. and Liu, J. (2006) *AAPS J.*, **8**, E729-E730.
- [6] Cromwell, E.M.; Hilario, E. and Jacobson, F. (2006) *AAPS J.*, **8**, E572-E579.
- [7] Hermeling, S.; Crommelin, D.J.A.; Schellekens, H. and Jiskoot, W. (2004) *Pharm. Res.*, **21**, 897-903.
- [8] Scherthaner, G. (1993) *Diabetes Care*, **16** (Suppl 3), 155-165.
- [9] Moore, W.V. and Leppert, P. (1980) *J. Clin. Endocrinol. Metab.*, **51**, 691-697.
- [10] Hjelm Skog, A.L.; Wadhwa, M.; Hassan, M.; Gharizadeh, B.; Bird, C.; Ragnhammar, P.; Thorpe, R. and Mellstedt, H. (2001) *Clin. Cancer Res.*, **7**, 1163-1170.
- [11] Li, J.; Yang, C.; Yuping, X.; Bertino, A.; Glaspy, J. and Roberts, M. (2001) *Blood*, **98**, 3241-3248.
- [12] Casadevall, N.; Nataf, J.; Viron, B.; Kolta, A.; Kiladjian, J.J.; Martin-Dupont, P.; Michaud, P.; Papo, T.; Ugo, V.; Teyssandier, I.; Varet, B. and Mayeux, P. (2002) *N. Engl. J. Med.*, **346**, 469-47.
- [13] Mire-Sluis, A.R.; Barrett, Y.C.; Devanarayan, D.; Koren, E.; Liu, H.; Maia, M.; Parish, T.; Scott, G.; Shankar, G.; Shores, E.; Swanson, S.J.; Taniguchi, G.; Wierda, D. and Zuckerman, L.A. (2004) *J. Immunol. Methods*, **289**, 1-16.
- [14] Geng, D.; Shankar, G.; Schantz, A.; Rajadhyaksha, M.; Davis, H.; Wagner, C.; (2005) *J. Pharm. Biomed. Anal.*, **39**, 364-375.
- [15] Klakamp, S.L.; Lu, H.; Tabrizi, M.; Funelas, C.; Roskos, L.K.; Coleman, D. (2007) *Anal. Chem.*, **79**(21), 8176-8184.

- [16] Gupta, S.; Indelicato, S.R.; Jethwa, V.; Kawabata, T.; Kelly, M.; Mire-Sluis, A.R.; Richards, S.M.; Rup, B.; Shore, E.; Swanson, S.J. and Wakshull, E. (2007) *J. Immunol. Methods*, **321**, 1.
- [17] Koren, E.; Smith, H.M.; Shores, E.; Shankar, G.; Finco-Kent, D.; Rup, B.; Barrett, Y.-C.; Devanarayan, V.; Gorovits, B.; Gupta, S.; Parish, T.; Quarmby, V.; Moxness, M.; Swanson, S.J.; Taniguchi, G.; Zuckerman, L.A.; Stebbins, C.C. and Mire-Sluis, A. (2008) *J. Immunol. Methods*, **333**, 1-9.
- [18] Swanson, S.J. (2004) *Nephron Clin. Pract.*, **96**, c88-95.
- [19] Ferbas, J.; Thomas, J.; Hodgson, J.; Gaur, A.; Casadevall, N. and Swanson, S.J. (2007) *Clin. Vaccine Immunol.*, **14**(9), 1165-1172.
- [20] Neyer, L.; Hiller, J.; Gish, K.; Keller, S. and Caras, I. (2006) *J. Immunol. Methods*, **315**(1-2), 80-87.
- [21] Brickelmaier, M.; Hochman, P.S.; Baciú, R.; Chao, B.; Cuervo, J.H. and Whitty, A. (1999) *J. Immunol. Methods*, **227**(1-2), 121-35.
- [22] Koskinen, J.O.; Vaarno, J.; Vainionpää, R.; Meltola, N.J. and Soini, A.E. (2006) *J. Immunol. Methods*, **309**(1-2), 11-24.
- [23] Park, M.K.; Briles, D.E. and Nahm, M.H. (2000) *Clin. Diagn. Lab. Immunol.*, **7**(3), 486-489.
- [24] Lofgren, J.A.; Dhandapani, S.; Pennucci, J.J.; Abbott, C.M.; Mytych, D.T.; Kaliyaperumal, A.; Swanson, S.J. and Mullenix, M.C. (2007) *J. Immunol.*, **178**(11), 7467-7472.
- [25] Morgan, E.; Varro, R.; Sepulveda, H.; Ember, J.A.; Apgar, J.; Wilson, J.; Lowe, L.; Chen, R.; Shivraj, L.; Agadir, A.; Campos, R.; Ernst, D. and Gaur, A. (2004) *Clin. Immunol.*, **110**, 252-266.
- [26] Patton, A.; Mullenix, M.; Swanson, S.J. and Koren, E. (2005) *J. Immunol. Methods*, **304**, 189-195.
- [27] Bourdage, J.S.; Cook, C.A.; Farrington, D.L.; Chain, J.S. and Konrad, R.J. (2007) *J. Immunol. Methods*, **327**(1-2), 10-7.
- [28] Lofgren, J.A.; Wala, I.; Koren, E.; Swanson, S.J. and Jing, S. (2006) *J. Immunol. Methods*, **308**(1-2), 101-8.
- [29] Wei, X.; Swanson, S.J. and Gupta, S. (2004) *J. Immunol. Methods*, **293**, 115.
- [30] Sadick, M.D.; Intintoli, A.; Quarmby, V.; McCoy, A.; Canova-Davis, E. and Ling, V. (1999) *J. Pharm. Biomed. Anal.*, **19**, 883.
- [31] Sakamoto, S.; Yokoyama, M.; Prakash, K.; Tsuruha, J.; Masamoto, S.; Getzenberg, R.H. and Kakehi, Y. (2003) *J. Biomol. Screen*, **8**, 701.
- [32] Wu, K.; Yang, C.; Davoli, M.A.; D'Amico, M.; Li, A.; Cveklova, K.; Kozmik, Z.; Lisanti, M.P.; Russell, R.G.; Cvekl, A. and Pestell, R.G. (2003) *J. Biol. Chem.*, **278**, 51673.
- [33] Yua, Y.; Piddingtonb, C.; Fitzpatrickb, D.; Twomeyb, B.; Xu, R. and Swanson, S.J. (2006) *J. Immunol. Methods*, **316**(1-2), 8-17.
- [34] Wala, I.; Swanson, S.J. and Jing, S. (2007) *J. Pharm. Biomed. Anal.*, **45**, 583-589.

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