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Review Article

Progress in the Use of Biological Assays during the Development of Biotechnology Products

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The complexity of the structure and function of many biotechnology derived products necessitates a wide range of analytical procedures to adequately characterize the product. In-depth characterization is required for the assessment of several criteria vital to the success of product development such as consistency, purity, stability, and potency. More recently, the concern over the immunogenicity of biologics has increased the need to develop assays to detect neutralizing anti-product antibodies. Although many physicochemical tests are available to characterize the structure of a protein and detect the presence of contaminants, they provide little, if any, information regarding biological potency or the neutralizing capacity of antibody responses in immunogenicity studies. There is a continual need to refine biological assays to increase their accuracy and reproducibility, in particular to replace *in vivo* bioassays with appropriate *in vitro* assays. There have also been several recent technological developments that could lead to more rapid and reproducible bioassays.

KEY WORDS: biotechnology; biological assays; immunogenicity; analytical; therapeutics.

INTRODUCTION

It has long been recognized that biological based therapeutic agents are substantially more complex than the majority of classical drugs both in structure and the relationship of structure to function. Whereas regulatory/scientific advisory bodies require a package of both biological and biochemical characterization of biological products for licensing, there has been some discussion over recent years about the role of biological assays in the development of biological products (1).

This has occurred in the light of increasingly sophisticated physicochemical techniques becoming available for protein sequence and structure analysis, such as nuclear magnetic resonance and mass spectrometry (2–5). However, it has generally been agreed that while such techniques are very valuable, they are as yet unable to predict the biological activity of the vast majority of biological products and therefore bioassays are an essential part of the characterization of the biological activity of any biotherapeutic (6).

In addition, there is currently an increased regulatory awareness for the need to assess the immunogenicity of biological products, requiring the development of suitable assays for the detection of anti-product antibodies (7).

DEFINING BIOLOGICAL ASSAYS AND POTENCY ASSAYS

Quantitating the biological activity of the majority of biological products can, to date, only be achieved by bioassay, where bioassays can be defined as "an analytical procedure measuring a biological activity of a test substance based on a specific, functional, biological response of a test system" (8). This contrasts with binding assays such as immunoassays that do not measure the ability of a protein to induce a biological response. The term bioassay should not be confused with potency assay. Potency is the ability of a material to exert its intended activity and may not necessarily have to be measured in a biological system. For example, antibody products that are intended to block the binding of one protein to another can have their potency measured in a binding assay. However, it is often the case that binding alone is not the sole biological endpoint of the product and a cell based format may provide a more relevant assay e.g., prevention of ligand binding to its receptor on the cell surface, where both a receptor based binding assay or a prevention of ligand induction of cell activity bioassay can be used. If a binding assay is chosen to measure potency, it is necessary to compare and validate the assay with a cell based assay, particularly for stability studies where the stability indicating properties of the assays must be assured (9). It is now often the case that both a binding assay and some form of cell based bioassay are required to characterize binding proteins, such as monoclonal antibodies and soluble receptors, with the choice of which assay to use for a lot release assay being dependent on the product and the assays available.

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ABBREVIATIONS: ICH, International Conference on Harmonization; KIRA, kinase receptor activation assay; SPR, surface plasmon resonance.

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SELECTING APPROPRIATE BIOASSAY FORMATS

It has been generally accepted that bioassays are a quality issue and that they should not necessarily need to be designed to predict or reflect any clinical efficacy per se, as this is the purpose of clinical trials. ICH guideline Q6b states that "mimicking the biological activity in the clinical situation is not always necessary. A correlation between the expected clinical response and the activity in the biological assay should be established in pharmacodynamic or clinical studies" (9).

However, biological products often have more than one biological activity. For example, cytokines can act on a variety of different cells and induce a number of biological responses ranging from proliferation to differentiation to cytotoxicity (10). Therefore, if several bioassays are available for a single product, should one choose an assay system that more closely reflects the intended use of the therapeutic?

A good example of this is in the case of interferon-alpha, a cytokine that possesses potent antiviral activity and is used clinically to treat viral infections such as hepatitis B and C (11). However, interferon-alpha also possesses immunomodulatory and anti-proliferative activity that is reflected in its use to treat diseases such as hairy cell leukaemia and bladder carcinoma. Bioassays for interferon-alpha can be based on its antiviral activity, where one uses its ability to protect cells from infection and destruction of by a selected virus. In addition, bioassays can measure the ability of interferon alpha to inhibit the proliferation of growth of certain cell lines. Therefore it remains debatable which bioassay one should use to assess the quality of this product.

If a product is only licensed for a single use that takes advantage of a single biological activity, it does seem sensible to use a bioassay that reflects this (e.g., erythropoietin stimulating the production of red blood cells, or Factor VIII inducing clotting). There is little evidence available that has shown definitively that one can dissociate different biological activities of a protein to different regions of the molecule. The general trend appears to be that changes in molecular structure, even small changes, can result in detectable changes in biological activity (12). If data does suggest that the structure of biologically active proteins may contain different areas devoted to exercising different biological activities, perhaps it would be valid to have more than one test for bioactivity, depending on the intended use of the molecule.

Because the bioassay is a quality issue and is used almost entirely to demonstrate batch to batch consistency, unless data is available to prove otherwise, any assay format is suitable as long as the assay selected is relevant, precise and robust (13,14). If the bioassay is to be used to assess stability then the stability indicating qualities of the assay need to be confirmed. Such data can be provided by accelerated or real time degradation testing, together with physicochemical analysis.

However, while biological relevancy is desired, economics may also be a factor in the choice of assay. An assay may well reflect the intended use of the drug, but may be expensive to run, time consuming and economically infeasible (Table I). Therefore a balance must be struck between the need to seek biological relevance, quality of the assay data, and the many other physicochemical tests available to characterize a molecule.

EXISTING AND NEWLY DEVELOPING BIOASSAY FORMATS

In Vivo Bioassays

Earliest attempts to measure biological activity often took the form of an *in vivo* bioassay, where protein was administered to animals and the response in those animals measured. Such assays included iron uptake and hematocrit in mice when administered with erythropoietin, growth of tissues when given sex hormones or the production of granulocytes when given colony stimulating factors (15,16). However, it is difficult to reduce inter-animal variability in estimates of potency, and *in vivo* bioassays are expensive, labor intensive

Table I. Comparison of the Characteristics of Various Assay Formats

Assay format	Throughput	Variability[a]	Special reagents	Time taken	Expenses
In vivo Bioassay	Low	High	None	Days-Weeks	Animal costs. Animal husbandry.
In vitro tissue based assay	Moderate	Moderate	Animal derived tissue	24–96 hours	Animal costs. Animal husbandry. Sterile laboratory facilities.
Cell line based assay	High	Low	Clonal cell line	24–96 hours	Sterile laboratory facilities. Plate reader.
Reporter gene assay	Very High	Low	Reporter gene transduced cell line	3–4 hours	Sterile laboratory facilities. Plate reader.
Kinase receptor activation assay	High	Low	Kinase receptor transduced cell line and antiphosphotyrosine antibodies	12 hours (Cell prep.) 10 minutes (kinase) 5 hours (ELISA)	Sterile laboratory facilities. Plate reader. Antibodies.
Biosensor cell binding assay	Low	Moderate/Low	Ligand binding cells and ligand. Biosensor chip.	20–30 minutes/sample	Sterile laboratory facilities. Biosensor equipment.

[a] Variability of a well controlled/designed assay.

(particularly animal husbandry) and often require the sacrifice of many animals to get statistically valid data. A balance must also be maintained between the large numbers of animals that could be used to provide several data points for potency estimates and humane, ethical, and economic pressures to reduce the use of laboratory animals for assays. It can be argued that testing *in vivo* provides biological potency tests more relevant to the clinical use of biologicals because a 'whole body' approach takes into account bioavailability, toxicities, etc. This argument is incorrect because biological assays are not intended to mimic the biological activity of a product in the clinical situation. As described, bioassays are intended to be used for quality control and illustrate the batch-to-batch consistency of biological potency of a product (17).

There may be a case to be made for *in vivo* testing where a combination of physicochemical and biological tests can not detect differences known to impact on *in vivo* activity. Such issues would involve complex glycosylation relevant to biological half-lives or modified protein products such as pegylated protein.

In Vitro Tissue Based Bioassays

Improvements to *in vivo* bioassays occurred with the development of *in vitro* bioassays, where cells or tissues from animals are cultured in the laboratory and used as responders to the test protein (16,18). In the case of cytokines, the majority of assays used cells from the haematopoietic system, with the earliest assays for colony stimulating factors using whole bone marrow as a source of haematopoietic progenitor cells. As cytokines were discovered with more mature cellular targets, subsets of haematopoietic cells from peripheral blood were used (19). Proteins that act on solid tissues, such as growth factors and hormones, require the removal of the specific tissue on which they act and its homogenisation into single cells that can then be cultured and exposed to protein *in vitro*. However, donor-to-donor variability still occurs in these systems and pure populations of target cells are difficult to achieve.

In Vitro Cell Line Based Bioassays

The development of clonal cell lines that respond to specific ligands is a significant improvement as a source of materials for bioassays (20). Malignant tumors can be dependent on a protein factor for growth and tend to be immortal, thus providing a single homogenous source of cells that are able to be distributed from laboratory to laboratory and can be used for assays. Such tumors are removed from source (most often murine or human) and homogenized into single cells. These cells are then grown in tissue culture, in the presence of the growth promoting protein, providing a continuous source of cells that then removes the need for any animals.

The cellular response of ligand-dependent cell lines can take a variety of forms, but is most often proliferation or inhibition of proliferation, expression of cellular markers or enzymes, cytotoxicity, or anti-viral activity (11,20). All these different bioassay systems have their disadvantages, most often lack of specificity. The use of murine cell lines increases specificity in some cases, as they may not respond to proteins that are species restricted in their activity. In addition, cells can respond to various other factors in biological samples (both inhibitory and stimulatory) and therefore it is recommended that an anti-ligand neutralizing antibody be used in assays to illustrate the activity of the material under test from amongst other influencing factors. This would also include the testing of interference due to the presence of biological matrix for bioassays used to monitor pharmacokinetics. Such experiments involve the spiking of test sample into various normal or patient matrix, such as plasma or serum, and calculating recoveries. However, if the bioassay is intended for the lot release of a well characterized biological product, this would not present a problem.

The advent of recombinant DNA technology has allowed for the cloning of specific receptors and their expression on previously non-responsive cell lines (11). This can create a specific, responsive cell line for almost any protein with a cellular receptor, without the need to screen a wide range of existing cell lines or tumor cells for responsiveness. It must also be stressed that one can not be assured that the removal and use of cells from tissues or the use of clonal cell lines in *in vitro* formats represents what is occurring *in vivo* and thus most, if not all, bioassays are a surrogate marker for biological activity.

With all cell line based assays, careful evaluation of the stability of the cell line should be carried out. Cell lines can often lose their biological responsiveness over time, so it is important to have a well-characterized cell bank and some idea of how long a line can be passaged before its response becomes compromised.

Reporter Gene Based Bioassays

While transfected receptor cell lines can offer selective responsiveness, such lines are still prone to the variability that occurs during the extended periods required for some induced biological function to appear (e.g., cell division, maturation or cell death). Therefore, the development of bioassays that identify the activation of the genes involved in that function can be much more rapid and robust. The format of these assays is to introduce a plasmid containing a promoter (or rather a relevant region) known to be involved in the expression of genes induced by a test ligand. The promoter region is linked to a reporter gene that subsequently gets expressed on ligand binding to its receptor (21,22). The earliest forms of such assays, termed reporter gene assays, used luciferase expression as a marker for gene activation induced by test ligands (Fig. 1). This enzyme catalyses a reaction that results in light formation detectable by luminometers. In recent years, even more sensitive reporter gene systems have been devised, including green fluorescent protein and beta-galactosidase (23,24). Due to the shorter time required for significant expression of reporter genes, 2 h as opposed to days for standard bioassays, the assays appear less affected by extraneous influences and are therefore less variable and more precise. However, as with any analytical technique, careful validation must be carried out to ensure that quantitative measurements are appropriate (25,26).

Kinase Receptor Activation Assays (KIRA)

Although the production of ligand responsive cell lines has been valuable in providing suitable *in vitro* bioassays for

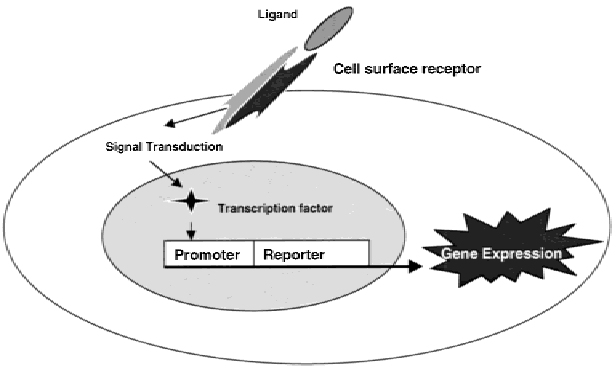

Fig. 1. An illustration of a typical reporter gene assay. A cell expressing ligand receptors is transfected with the promoter region of a gene known to be induced by the ligand. The promoter is attached to a reporter gene that is then expressed on ligand binding to its receptor. The amount of reporter gene expressed is proportional to the quantity of ligand binding to the receptors on the cell surface.

a significant number of protein products, for some protein products there is no suitable cell line based bioassay that is appropriate for reproducible routine use. In this case, development of receptor-based 'biochemical bioassays' are being developed. While at an early stage of development such assays have been designed for cytokines and growth factors that posses an integral enzyme in their receptors and where enzyme activation can be used as a marker for ligand levels (27). The binding of some cytokines and growth factors to their receptor/s on cell surfaces induces the rapid phosphorylation of the receptor/s on tyrosine residues. The cells are then lysed and an immunoassay is used to detect the level of phosphotyrosine being produced (Fig. 2) (28). The level of tyrosine phosphorylation is a correlate of ligand binding and receptor activation. The KIRA assay phosphorylation step takes only 5–10 min and allows for the assay of products under conditions that would normally interfere in standard format bioassays e.g., in serum or plasma.

As these assays are quite rapid and reproducible, they are of greatest value for proteins where simple cell line based bioassays do not exist, as they still require the culture of cells/cell lines and have the additional step of an immunoassay. In addition, how transfected receptor phosphorylation relates to overall biological activity also requires continued investigation. These assays will be of most use when non-enzyme linked receptors can be cloned to include a marker enzyme and replace awkward or non-existent bioassays (e.g., the chemokine receptors to replace chemotaxis assays).

Biosensor Based Binding Assays

Most, if not all, biological products act through some form of binding to another moiety. Antibodies bind to their antigens, cytokines, growth factors, and hormones bind to cellular receptors and enzymes bind to their substrates. Therefore, as previously discussed, it has been an issue for some time as to whether simple binding assays can replace bioassays. However, immunoassay format binding assays have several disadvantages since they are unable to reproduce the binding conditions on a cell surface as antigen or receptor protein is coated onto a plastic surface. It is known that adherence of proteins to plastic surfaces can alter their structure

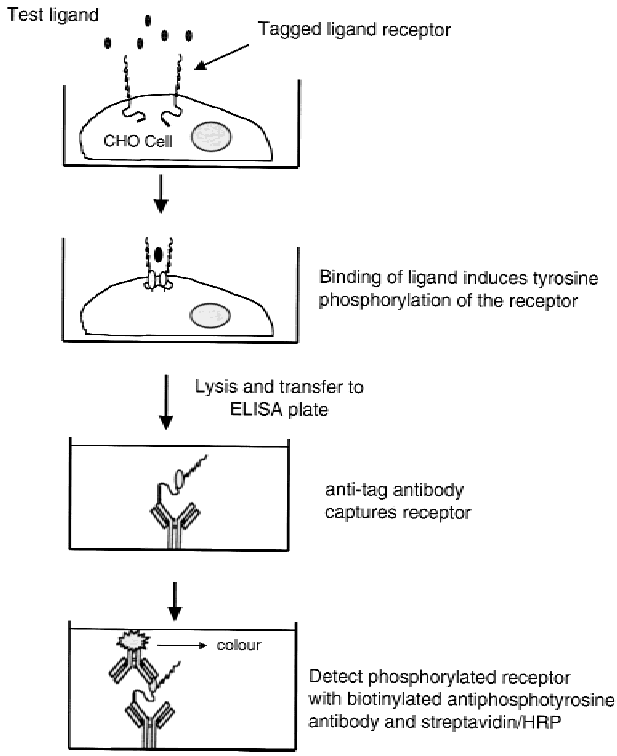

Fig. 2. The Kinase Receptor Activation Assay. Chinese hamster ovary (CHO) cells are transfected with the ligand receptor of choice attached to a tag. Addition of ligand to these cells results in enzymatic activation of the receptor to autophosphorylate itself on tyrosine residues. The level of phosphorylated receptor is measured by immunoassay and is used as a marker of the level of ligand added to the original cells.

and therefore most likely their binding capabilities (29). In addition, little information is provided by immunoassays on the kinetics of the binding activity. Recently there has been extensive development of a binding platform based on the ability of ligands binding to molecules embedded in a dextran mesh on the surface of a gold plated sensor chip to elicit changes in surface plasmon resonance that is detected as changes in reflected polarized light (Fig. 3) (30,31).

Surface Plasmon Resonance (SPR) occurs in a thin metal

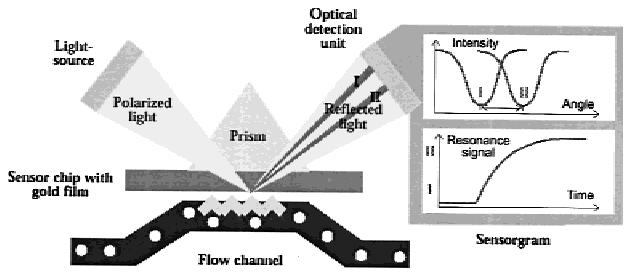

Fig. 3. An illustration of the use of surface plasmon resonance to measure binding phenomena using the BIACORE® format. Polarized light is targeted onto the surface of a thin gold film on a sensor chip. At a specific angle, absorption of light photons results in their conversion to surface plasmons and an electrical field. Binding phenomena occurring on the other side of the gold film alters the electric field/plasmon velocity and the angle at which light conversion occurs. This is detected by an optical detection unit and expressed as a sensorgram.

film at an optical interface under conditions of total internal reflectance and is observed as a decrease in the reflected light intensity at a specific angle. When molecules bind to the surface of the film (or in this case ligands immobilized on its surface), this causes changes in surface plasmon velocity resulting in a shift of the incident light angle at which resonance occurs. The shift in angle of reflected light is detected by light sensors and illustrated on a sensorgram.

This technology enables molecules to be imbedded in a carboxymethyl dextran layer that forms a type of hydrogel. Therefore the molecules are not stuck onto plastic nor require labeling (e.g., with enzymes or fluorescent labels) and thus binding epitopes are not altered or masked. In addition, full kinetic parameters such as on and off rates and binding affinity can be assessed.

Several biotechnology products have had their binding properties examined using this technology and validation parameters for use of the technique for product analysis have been defined (32–34).

SPR technology is rapidly advancing and is now able to detect the binding of cells to immobilized ligands (35). This is particularly informative as cell surface receptors are left in an unadulterated state and represents a highly 'natural' binding format and allows for the use of living cells to provide a form of biological assay based on binding to a cell surface.

THE ROLE OF BIOASSAYS IN ASSESSING IMMUNOGENICITY

There has been increasing regulatory concern over the immunogenicity of biological products and a focus on the design and interpretation of assays to detect antibodies raised against biological therapeutics. The primary concern with immunogenicity for manufacturers, regulatory agencies, and clinicians is whether the presence of antibodies produced by patients receiving a product results in clinical sequelae. However, the assessment of this concern is entirely dependent on the appropriate detection, measurement and characterization of the antibodies. Probably the most significant safety issue regarding antibody formation in the patient would be the production of neutralizing antibodies that cross-react and neutralize endogenous counterparts after the treatment is over, resulting in long term adverse events.

Immunoassays used for antibody detection cannot generally predict whether a positive antibody preparation will contain antibodies that can neutralize the biological activity of the product. In addition, standard immunoassay formats are prone to a wide number of interference challenges and the washing steps necessary to address them tend to favor the detection of primarily high affinity antibodies (7). More recently however, the use of SPR technology to detect antibody binding to its ligand has resulted in the ability to both detect low affinity antibodies and more thoroughly characterize the antibody response. Even though SPR technology is a useful advance in the detection of anti-product antibodies, it is unable to predict if a binding response results in loss of biological activity (36). Therefore, the neutralizing capacity of antibodies to most products require testing in a biological assay. However, bioassays can be exquisitely sensitive to matrix effects and care must be taken to confirm that any inhibitory activity in the bioassay is due to specific antibodies and not matrix. The use of the KIRA technology described previously allows for a more rapid assessment of neutralizing activity and such assays are less prone to the inhibitory affects of biological matrices (28).

THE USE OF BIOLOGICAL STANDARDS AND UNITAGES

Because different manufacturers' products, even of the same product from the same cell source, can possess very different specific activities, mass cannot be used as a measure of the functional activity of a biological material. In addition, mass cannot be used because a degrading protein may have the same mass, but possess altered biological activity. Therefore, a unit has to be defined for such activity associated with a characterized reference standard (37).

Because bioassays are a quality issue, the use of a potency unit applied to a product should be treated accordingly and not be used to dose similar unitages of different products with the assumption that they will induce the same clinical response. For the majority of biological products, where absolute identity is difficult to establish, it is the case that only when two products have been compared in the same (or possibly similar) clinical trial/s can any assumptions about interchangeability of dose be made (38).

Therefore, if a unit has to be defined for such activity without a single reference preparation, units with different definitions can occur and cause confusion, leading to substantial interlaboratory differences in estimates of potency. The concept of a single internationally available potency standard has been developed under the auspices of the World Health Organization and shown to be extremely valuable in reducing the laboratory variability in potency estimates and to enhance comparability of clinical and research studies. The World Health Organization has for many years established well-characterized potency standards calibrated in International Units that are available for use in the pharmaceutical and research community.

To establish WHO International Standards, a rigorous procedure is followed according to WHO guidelines to ensure that the appropriate material is selected to serve as a standard. However, this process is quite lengthy and therefore in the rapidly developing area of cytokines and growth factors a new category of standards, the WHO Reference Reagent (RR) has been established. An RR is a standard prepared to WHO guidelines that is checked by the collaborating center that prepared it and the supplier of the material. The standard is monitored so that no loss of activity occurs during lyophilization and that biological activity remains stable. This allows for companies to approach a WHO collaborating center as early on in the development of the product as possible and work together to establish an RR and appropriate unitage. This prevents the unnecessary effort of companies having to establish in-house units and then having to alter unitages on establishment of a WHO Standard.

The role of the biological potency unit in biotherapeutics has been the subject of some confusion and led to a meeting at the National Institute for Biological Standards and Control in 1997 between scientists, regulators, and manufacturers, to discuss the purpose of the potency unit and role of bioassays in the development of biotechnology products. It was agreed that as bioassays are a quality issue (as discussed previously) and that the potency unit applied to a product should be

treated accordingly. There is no guarantee from *in vitro* assays that any two products will behave the same *in vivo* in man; this is the role of clinical trials and therefore, it can not be assumed that one unit of one product will act the same *in vivo* as one unit of another. For example, some glycosylated products can have the same specific activity as non-glycosylated counterparts *in vitro,* yet be significantly more potent *in vivo* where glycosylation can increase blood half-lives and stability (e.g., erythropoietin). However, many biological products are dosed on units (e.g., insulin) and it is semantics how one calibrates dosage (units, mass, vials, etc.), but it is the incorrect assumption that one can interchange similar products on a unit basis that must be avoided.

The choice of what unitage to use for any material is part of the collaborative study process, but is essentially an arbitary unitage, defined solely by the WHO standard. It had been the case that biological materials were calibrated against some aspect of the bioassay used to measure potency (e.g., ED50's), but this has been proved to be invalid because the performance of bioassays (and hence their biological readouts) can vary from day to day and especially from laboratory to laboratory. Therefore, the use of a single reference preparation, with its associated unitage, allows multiple laboratories using different biological assays to compare their in house material to the standard and calibrate it accordingly.

The other role of assigning a potency unit related to a reference preparation is a regulatory one. International unitages and standards are vital if the biological potency of any preparation is to be assessed. Stating mass is no help as one cannot possibly weigh a drug supplied, particularly if it contains excipients, as most biotherapeutics do. If there is a perceived problem with the product and its activity needs to measured by a control authority or other third party, a reference material is required.

DESIGN AND ANALYSIS OF BIOASSAYS

The design of any bioassay must take into account factors that introduce variability and therefore the analysis of bioassays must test for variability if results are to be statistically valid. Although the design of *in vitro* bioassays can take many forms, an assay that relies on a determination of quantity based on a single dilution of a test material is decidedly not valid. A titration of the test material has to be made and compared to a titration of a reference material, with particular attention paid to comparisons of the linear portion of the dose-response curve (1) (Fig. 4). At least three points on the linear portion of the dose response curve are required to compare sample and reference curves.

Carefully controlled bioassays are technically demanding, relying heavily on the competence of staff carrying out the assays to accurately and reproducibly dilute and pipette solutions. However, attempts to automate bioassays have been particularly successful when such tasks have been taken over by robots, but the capital investment is large and therefore this has not come into routine use by the majority of manufacturers. The development of the *in vitro* bioassay techniques described has led to the increased use of microtitre-plate-based assays. These assays are particularly prone to position effects that can result in variability of data. However, although it is extremely difficult to use an assay format that suffers from such effects, the knowledge that they occur allows for efforts to identify and resolve position effects before bringing any assay into routine use. To reduce the effects of position within microtitre-plate assays, randomization of the position of sample titration curves within plates is recommended, as is the inclusion of a standard reference preparation on each plate; again, preferably in different positions (39,40). The use of coded duplicates in the assessment of variability and bias is particularly valuable.

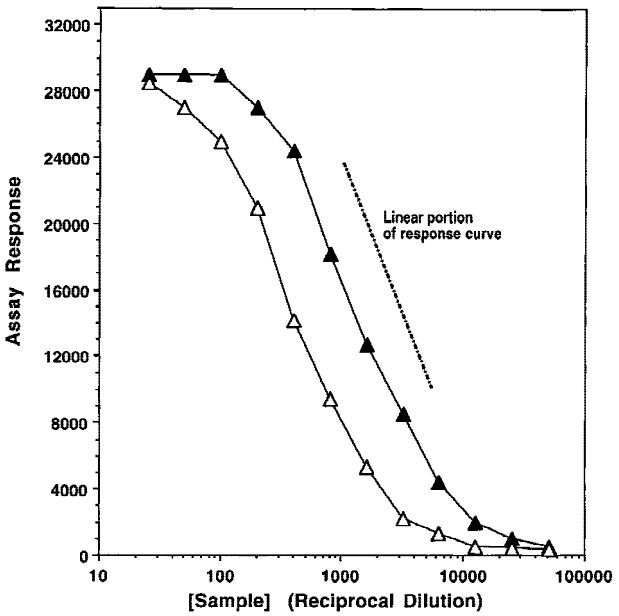

Fig. 4. An example of a bioassay using dilution series of a reference preparation (△) or test material (▲), illustrating the parallel line portions of the dose response curves. The x-axis represents the dilution of test and sample preparations and the y-axis the measurable response of the assay. The relative potency of the two preparations is calculated from the distance between the parallel portions of the curves.

Methods for the statistical analysis of tests such as bioassays are included in the various pharmacopoeias, but only state statistical concepts, as each laboratory will have different programming packages and data sets (41). Setting limits around the mean and fiducial limits for bioassays has particular importance for biologicals. While the former is a test of the batch to batch consistency of the product potency, the latter is a test of the variability of the bioassay (1,6).

The appropriate validation of any bioassay used for the characterization of biological products is critical. Even though there are general guidelines for assessment of the validity of an assay (9,13,14), these are not specific to biological assays. Therefore, it is up to the bioassay developer to use these guidelines and develop in house protocols based on sound scientific principles and the nature of the assay. Further discussion of the validation of bioassays is, unfortunately, beyond the scope of this review.

CONCLUSION

The development of accurate and well-characterized biological assays for biological products is vital for their development as therapeutic products. Whereas a highly sophisticated physicochemical tests exist that can provide a great deal of information about their structure, only bioassays can mea-

sure biological activity. The use of animals for the measurement of activity has greatly diminished with the advent of dependent cell lines, although a need for *in vivo* assays may remain in cases where existing *in vitro* assays cannot detect differences that may impact on *in vivo* activity. New technology is increasingly being used, such as reporter gene assays and surface plasmon resonance binding assays, making bioassays more reliable and robust. The increasing awareness of the role and importance of biological assays in product development is leading to assays that are more appropriately designed and analyzed, thus providing higher quality data for the characterization of the product.

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