

Successful Strategies to Measure Residual Host Cell Proteins

Residual Host Cell Protein (HCP) assays are an essential requirement for the characterisation of biologics and vaccine products and frequently appear in the release specifications of products. Regulators have specified that the amount of HCP be controlled and measured from the very earliest stage of clinical development due to the potential for these contaminants to affect the potency of drugs and cause side effects. HCP, as the name suggests, originate from the cells used in the manufacture of product and can be considered as an impurity. These proteins can be very complex in nature and will vary in their constituency as product moves from the very crude bulk harvest material to the final drug bulk and product. HCP assays are performed on all bacterial, yeast and eukaryotic cells.

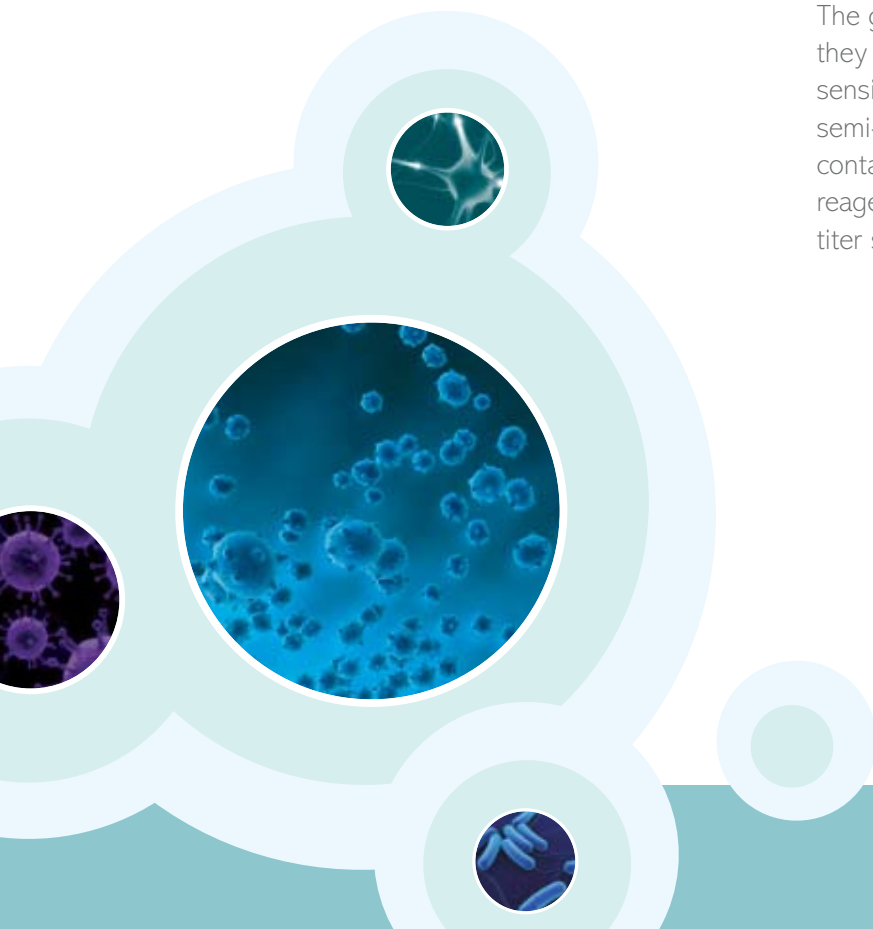
Basic ELISA Technologies

www.cygnustechnologies.com

During very early stages of product development it has been the custom to use “off the shelf” solution for the measurement of HCP. The company Cygnus Technologies, based in USA, manufactures ELISA kits for all of the most common HCP used in the production of vaccines and biologics.

These include :- Human A-549 cells, Baby Hamster Kidney cells, Chinese Hamster Ovary Cells, Human Embryonic Kidney 293 cells, Human Embryonic Lung MRC-5 cells, Murine NS0 cells, the primate cells line Vero, insect cell line SF-9, the yeasts *Pichia* and *Saccharomyces* and the bacteria *Pseudomonas* and *E.coli*.

These kits are widely used and provide sufficient data to be acceptable during early phase of drug development. The generic ELISA kits are widely popular because they are readily available, easy to use and highly sensitive. The kits are intended to be used for the semi-quantification of Host Cell Proteins and bioprocess contaminants. The kits are complete with all of the reagents necessary to perform the assays on a micro-titer strip well format.



Sample Qualification and Assay Validation

BioOutsource has already acquired data on selected Cygnus kits to demonstrate the utility of these assays in our hands; however it is essential to monitor these kits for their suitability with individual client samples. BioOutsource also has the ability to offer these assays for the cGMP batch release of product in Europe and USA.

BioOutsource has created a generic study protocol which will provide a comprehensive qualification of a sponsor specific sample type in the assay of choice. Our study protocol is based on the current ICH guidelines for the validation of analytical methods. The first experiments in the protocol will be to qualify the sample type for use with the Cygnus kit by spiking known amounts of reference standard HCP into the sponsors sample under test and assess the results of the sample both with and without the spike. This spiking experiment can be performed over the expected concentration range of HCP in the sample, if known, and can be performed with 4-6 concentrations of spike, typically performed in triplicate. The manufacturer suggests that a recovery rate of 80-120% of the spike amount can be expected, however in our experience this is very dependent on sample type and preliminary experiments are recommended to determine the suitability of sample type.

The HCP assays already have a Limit Of Quantitation (LOQ) and Limit of Detection (LOD) when the reference standards are tested in buffer and this is specified by the manufacturer. These are LOD = 400pg/mL to 1.1ng/mL for the CHO HCP assay and LOQ = 450pg/mL to 1.4ng/mL for CHO HCP assay. It would be expected that the experiments would include samples spiked with concentrations near the LOD and LOQ to determine the recovery expected at these concentrations in the sample matrix.

A second important consideration to these assays is dilutional linearity. This assessment ensures that, should

samples be diluted during processing, the diluted sample will yield results which are directly proportional to the extent of dilution. This assessment involves diluting the reference standard protein which is supplied by the kit or will reference provided by the sponsor through a number of serial dilutions in the sample matrix and buffer. These dilutions are then assayed and the HCP concentration determined. The linearity can be established by regression analysis to determine the derived equation for the dilution compared with the determined concentration. The closer this relationship is to linear ($r^2 = 1$) the more confidence there is that the assay is linear. Typically in BioOutsource we would expect to see an r^2 of greater than 0.90 over the expected range of the assay.

The last part of qualification of these assays is to determine the precision of the assay, depending on how the spiking and dilutional linearity experiments have been performed there may be sufficient data to establish the confidence in precision with the assay. Precision experiments are performed by the same technician with the same sample on different days. This establishes the variability of the assay using different pieces of equipment or different batches of reagent. BioOutsource would normally expect the variability of the assay to be less than 20%. Any higher than this and an assessment of the assay performance would be under question.

Advanced HCP Assays

Following early stage clinical trials, the regulatory requirements for HCP assays become more stringent and there is the requirement to develop product specific HCP assays rather than relying on the generic assays. The development of a product specific assay requires an understanding of the potential proteins which could be present and contaminating in the final product and to identify immunological reagents which are capable of detecting these proteins. In Europe the regulation produced by the EMEA entitled ICH Topic Q 6 B Specifications: Test Procedures and Acceptance

Criteria for Biotechnological/Biological Products. Note For Guidance On Specifications: Test Procedures And Acceptance Criteria For Biotechnological/Biological Products (CPMP/ICH/365/96) finalised in September 1999 stated

“Process related impurities, i.e., cell substrates (e.g., host cell proteins, host cell DNA), cell culture or downstream processing. Product-related impurities (e.g., precursors, certain degradation products) are molecular variants arising during manufacture and/or storage, which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety. Further, the acceptance criteria for impurities should be based on data obtained from lots used in preclinical and clinical studies and manufacturing consistency lots. Individual and/or collective acceptance criteria for impurities (product-related and process-related) should be set, as appropriate.”

It is possible that the commercially available immunological reagents would be suitable to detect the host cell contaminants from cells such as CHO or Murine NS0, however previous experience has shown that the modification of cell lines to produce a product does affect the profile of proteins expressed by the cell and this requires specific reagents to be created. Furthermore, the specific host-cell protein profile is likely to be highly dependent on the design of the manufacturing process. It is frequently necessary therefore to evaluate the proteins which are present in a production process and to determine if the reagent available will detect these in their entirety. In most cases, manufacturers find it necessary to develop a process and product-specific HCP assay for use during batch-release testing for Phase III clinical trial material and licensed product.

The first step in HCP evaluation is normally to acquire a protein profile of the contaminants. This can be relatively easily accomplished using standard polyacrylamide gel techniques with appropriate visualisation methodologies such as silver staining. A western blot is performed to measure the extent that the immunological detection reagents will pick up the different HCP seen in the gels. Following western

blotting the extent of the binding of the detection antibody to the proteins detected can be established. If the stained protein profile mirrors the western blot profile then this should provide sufficient data to ensure that all proteins present will be detected. If there are proteins absent from the blot then consideration should be made to create a new antiserum for detection of proteins. It will be the case that some proteins will be too small to elicit an immune response and therefore may not be detected in these assays.

Should the development of a product specific HCP be required, there are typically two reagents within a sandwich ELISA format. The first is the binding antibody and the second is the detection antibody to which a marker molecule is conjugated. HCP detection relies on the binding antibody being attached to a micro-titre plate, upon addition of the sample the binding antibody will bind and hold any HCP in the sample. Following washing procedures any unbound proteins are removed and the detection antibody added. The detection antibody will bind to the HCP held by the binding antibody and washing and addition of an appropriate substrate for the marker molecule then takes place. Conversion of the substrate indicates the presence of HCP. Reference standard material can be used to gauge the level of converted substrate in comparison to the amount of HCP present.

Alternative Techniques to ELISA Technology

Threshold System

www.moleculardevices.com

There are alternatives to the standard ELISA technology. One method which has been used for a number of years is the Threshold method, manufactured by Molecular Devices. This technology utilises the same antibody detection methods as the ELISA technique but has the advantage that the reaction takes place in the liquid phase rather than bound to the micro-titre plate which may affect the stoichiometry of the reaction. The binding of HCP is identified by a urease conjugated to the detection antibody which converts

the substrate urea to ammonia and carbon dioxide. This is then detected by a pH change. The sensitivity of this method is reported to be at least 4ng of HCP per mg of product.

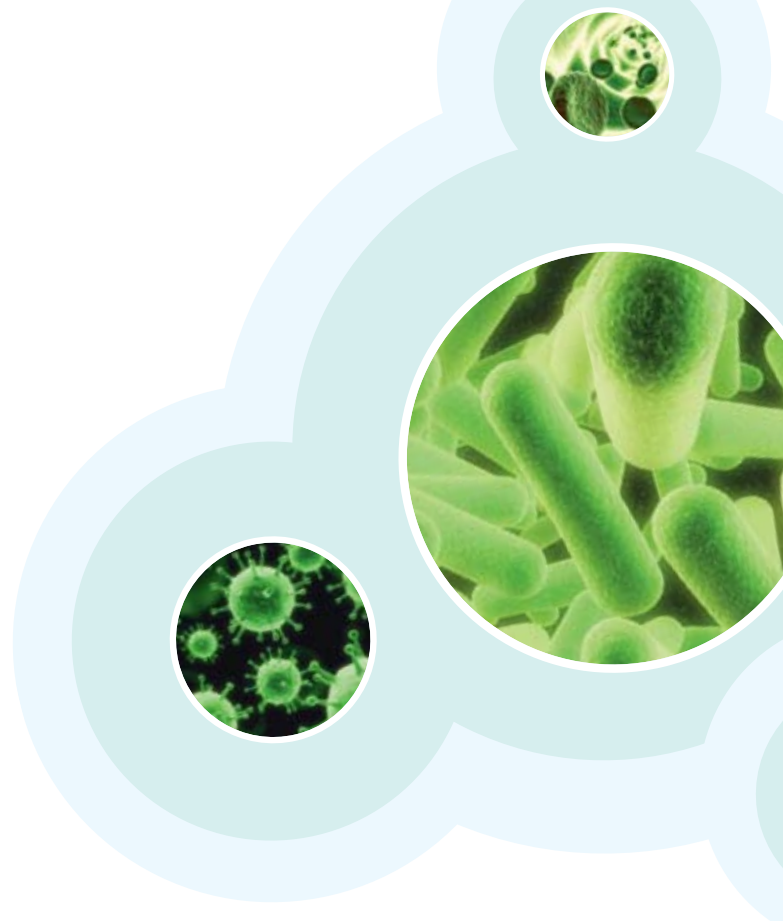
Meso-scale Discovery Technology

www.meso-scale.com

A second alternative to the ELISA technology is the Meso-scale discovery technology which is based on the replacement of the marker on the detection antibody with a Ruthenium ion which allows the electro-chemiluminescence to detect the presence of HCP. This method is reported to achieve a sensitivity of at least one log and possibly two logs of increased sensitivity over ELISA assays.

Summary

HCP determination is always critical during the development of a new drug. In drug substance it should be below detectable levels using a highly sensitive analytical method, usually less than 100ppm. However no exact limit for HCP can be established because of the differences in production processes regimes and clinical dosing regimes. The specificity and sensitivity of any antibody-based assay used for detection of HCP is directly related to the quality of the antibodies used to detect the proteins themselves. The goal of the assay is to detect the variety of different proteins that represent the HCP spectrum of the process and the product. Any immunoassay used to measure HCP should be evaluated and proven capable of reporting the true extent of HCP contamination. Validation of such assays will follow the ICH guidelines already discussed. Developing such an assay can require a considerable amount of time, principally due to the length of time taken to acquire the antiserum. However it should also be noted that, should there be any change in the production process, then the HCP assay would require re-evaluation and possibly further development.



BioOutsource Service Offering

BioOutsource offers a complete service for the detection of Residual Host Cell Proteins. Where appropriate, we can offer the detection of HCP using all of the available Cygnus Technologies kits. These tests are performed to cGMP standard and are suitable for testing early batches of clinical trials material.

For more advanced projects, BioOutsource offers the complete development of product and process-specific HCP assays from the immunisation of the animals with the target proteins to the qualification and validation of the assays to ICH standards.

Please contact us at alewin@biooutsource.com for more information.