High Throughput Process-related Impurity Testing Using a Gyrolab™ Workstation

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Abstract

In biologics manufacturing, it is necessary to accurately quantify process-related impurities to monitor the performance of the manufacturing process, and to measure the level in the Drug Substance. Typically, process-related impurity testing is performed using a sensitive immunoassay method such as ELISA. Although this method is sensitive, it has the disadvantage of being labor and reagent intensive, and is not scalable for large numbers of samples.
This poster describes the development of a Gyrolab Workstation method to quantify process-related impurities, and compares its performance to an established sandwich ELISA. In addition, advantages of the Gyrolab Workstation over the ELISA platform will be discussed.

Introduction

Process-related Impurities
Quantification of some process-related impurities (e.g. host cell protein) can be challenging as they are complex mixtures of proteins that require polyclonal antibodies for detection. Historically, quantification of process-related impurities was performed using sandwich ELISA methods. The Gyrolab Workstation provides an alternative platform to ELISA based assays that is similar to, but offers several advantages over, the ELISA platform.

Similar Method Development
A Gyrolab Workstation method is similar but simpler to develop than a standard sandwich ELISA method. Both require a standard curve prepared from an antigen that is representative of the process-related impurity. Polyclonal antibodies are evaluated and selected ensuring adequate coverage of the impurity. Both methods require a simple labeling of the polyclonal antibody. Figure 1 represents a comparison of the Gyrolab Workstation and ELISA platforms.

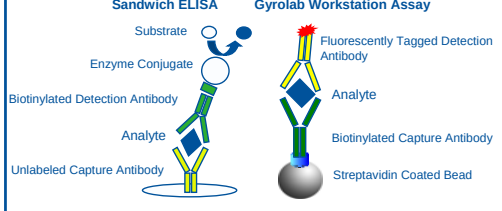

Figure 1: Comparison of sandwich ELISA and Gyrolab assay platforms

Simpler Method Development
The development of the Gyrolab Workstation method is simpler than an ELISA method. In an ELISA method the capture, detection and conjugate concentrations must be optimized, whereas a Gyrolab Workstation method only requires the detection antibody concentration to be optimized. The Gyrolab Workstation method does not require plate coating, manual wash steps or incubations. By using a fluorescent detection antibody rather than an enzyme conjugate, the standard curve range is increased by 2 to 3 orders of magnitude beyond ELISA. Multiple optimization assays can be performed in a single day using a Gyrolab Workstation, in contrast to an ELISA method that can require days or even weeks of assays to be fully optimized.

Gyrolab Workstation Technology
The Gyrolab Workstation platform uses compact disc (CD) microlaboratories in place of multiple well plates in the ELISA platform. Figure 2 details the structure of a Gyrolab Workstation CD microlaboratory.

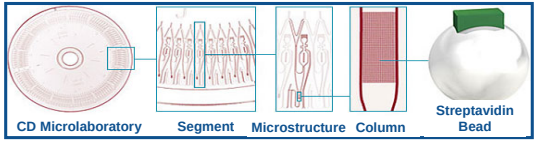

Figure 2: CD Microlaboratory structure

A microstructure is similar to a well in an ELISA plate. A CD microlaboratory rapidly flows reagents and analytes through columns rather than a surface interaction requiring incubation in an ELISA well, resulting in shorter assay run times. The Gyrolab Workstation has an internal laser and detector and automated software to evaluate sample results.
Samples and reagents are manually loaded onto the microplate carousel and automatically transferred to the CD microlaboratories by the robotic arm. At the CD spinning station the analytes are pulled through the CD microstructures by centrifugal force. After all reagent and analytes are applied the CD is transferred to the Laser Induced Fluorescence (LIF) Detector where data is obtained. Refer to Figure 3 for a diagram of the Gyrolab Workstation

Method Comparison

Antibodies
The same purified polyclonal antibody is used in both the ELISA and Gyrolab Workstation methods. Reference Figure 1 for ELISA and Gyrolab reagent requirements.

Antigen
The same antigen is used in both Gyrolab Workstation and ELISA methods

Samples
Samples are taken at different stages in the purification of a therapeutic protein. As a group they demonstrate the range of process-related impurity concentrations that can be expected in routine sample testing. The samples were prepared independently and tested using the Gyrolab Workstation and ELISA platforms.

ELISA
A high-binding 96 well microplate was coated overnight with unlabeled polyclonal antibody. The plate was washed and blocked, and after blocking the plate was washed again. The standard curve, samples and controls were diluted and loaded into the 96 well plate. Following incubation and another wash step a biotinylated polyclonal antibody was added and incubated. A streptavidin-enzyme conjugate was then added after another wash step and incubated. The plate was washed again and a colorimetric substrate was added and incubated. The reaction was stopped and the absorbance read. Raw data were analyzed by regression software then adjusted results were manually calculated.

Gyrolab Workstation
Standard curve, samples and controls were diluted in proprietary Gyros buffer and loaded into a sample microplate. The capture and detection antibodies were diluted and loaded into the reagent microplate along with a needle wash buffer. Prior to commencement of the assay the Gyrolab instrument is primed with wash buffers. Once the CDs and microplates are loaded on the Gyrolab the execution of the assay and interpretation of results were fully automated.

Figure 3: Gyrolab instrument diagram

Data Comparison

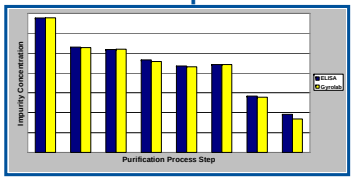

Figure 4: Comparison of Gyrolab Workstation and ELISA results through the purification process steps of a therapeutic protein. Process-related impurity results are comparable at both high and low concentrations.

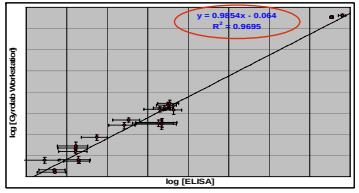

Figure 5: A plot of $\log_{10}$[Gyrolab Workstation results] vs $\log_{10}$[ELISA results] for samples throughout the purification of a therapeutic protein. The R^2 value and slope of the line generated are both nearly 1, indicating excellent correlation of results between the two methods. The error bars indicate less variability of the results obtained using the Gyrolab Workstation.

Conclusions

Compared to the ELISA method the Gyrolab Workstation method:
- Increases sample throughput while providing equivalent results
- Requires less analyst time
- Consumes less sample and reagent
- Requires fewer retests due to a wider dynamic range

Acknowledgements

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