Assessing the Performance of the ViBE Platform in Three Key BioProcess Assays – Host Cell Protein (HCP), Protein A Contamination and Product Titer Measurement

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Abstract

Biopharmaceutical manufacturing quality and process control requires contamination testing and purity assessments at critical stages in production to avoid compromising patient safety and the efficacy of the final drug product. Typical host cell related impurities are HCPs, nucleic acids, endotoxins, and viruses. Process-related impurities include cell-culture reagents or raw materials used during downstream processing and purification (ie. Protein A)

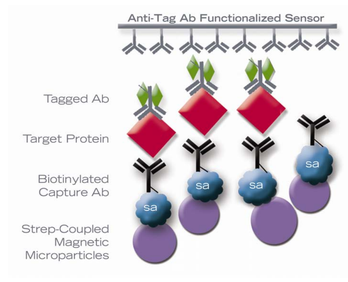
The purpose of this work is to evaluate the performance of newly emerging technology: the ViBE Workstation and AMMP assays on three key bioprocess testing applications. Using an assortment of bioprocess samples selected from multiple stages of the purification process, the feasibility of the ViBE workstation was tested for CHO-HCP and Residual Protein A contamination and product titer measurement. The ViBE performance was evaluated for reproducibility, precision and accuracy. HCP results demonstrated comparability with established ELISA techniques using 102 samples. Protein A contaminant reproducibility results showed single digit CVs while completing 96 samples within 2.5 hours, completely unattended. Product titer data were verified in "no-spin" samples containing over 10 million cells within 10 minutes and regardless of cell viability.

Results: These data show that the ViBE produces results comparable to established techniques yet offers significant process workflow advantages.

Technology and Instrumentation

BioScale's ViBE Bioanalyzer and ViBE Workstation powered by Acoustic Membrane Microparticle (AMMP™) technology were assessed in the protein analysis of three bioprocess assays. Comparison data using traditional immunoassay methods such as ELISA was used to determine comparability.

AMMP is the unique integration of magnetic microparticles, a universal sensor and acoustic detection creating a protein detection technique that is highly robust and versatile. AMMP assays use a traditional homogeneous assay format with a proprietary detection technology providing results with fewer sample limitations, simple operation and fast assay development.



Host Cell Protein Assays

The performance of the ViBE Protein Analysis Platform powered by the Acoustic Membrane MicroParticle (AMMP™) assay was evaluated compared with a commercially available ELISA for Chinese Hamster Ovary (CHO) HCP contamination. An assortment of bioprocess samples were selected from multiple stages of the purification process to demonstrate these comparison results.

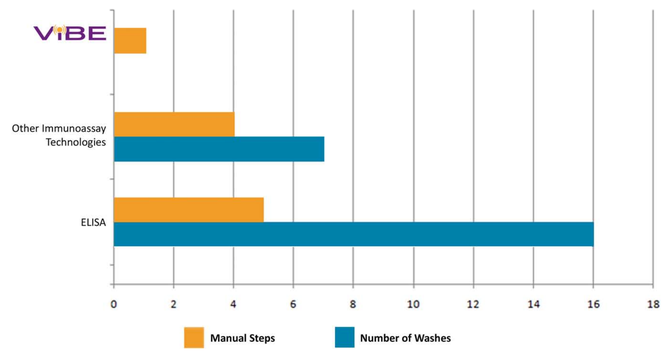
ViBE Workstation performance was evaluated for inter- and intra-assay precision, quantitation range, and the ability to accurately measure the concentration of controls spiked into a sample. Both measurement techniques were able to accurately measure the concentrations of CHO HCP contaminants in 102 bioprocess samples. The results agreed well across the entire quantitation range, with a 3 ng/mL average difference between the two data sets. These data show that the ViBE Workstation produces results comparable to established techniques and offers significant advantages with respect to work-flow and hands-on time.

ELISA versus ViBE

A control sample was run at two dilutions on a commercially available ELISA assay and the ViBE workstation. The data was compiled from eight separate assays run over a time period of several weeks. The ViBE workstation produced similar results to the ELISA with coefficient of variation as good or better than the ELISA assay.

Comparative Performance — ELISA vs. ViBE data for eight samples run over several weeks. The ViBE system shows a similar response with C.V.'s as good as or better than the commercially available ELISA assay.

	ELISA		ViBE	
Dilution	1:5000	1:10,000	1:5000	1:10,000
Average	52.8	23.3	69.4	30.2
SD	8.5	3.0	4.8	3.8
CV	16%	13%	7%	13%



Reduced Hands-on-Time

The BioScale ViBE platform with AMMP technology reduced the number of manual steps (minimizing operator error and operator hands-on time) in the HCP analysis workflow by replacing traditional, manually intense methods.

Assay Performance

When compared to an ELISA assay, the ViBE workstation using the AMMP CHO-HCP assay gives similar results with respect to accuracy and precision of CHO-HCP contaminant detection. Assay performance on the ViBE system was assessed for repeatability by measuring three standards of known concentration twenty times on one ViBE HCP cartridge. The results reveal intra cartridge coefficients of variations (CV's) of 10% or less for the AMMP CHO-HCP assay. Assay reproducibility was assessed by running three standard CHO-HCP samples in duplicate across three lots of cartridges on three separate days.

Repeatability- three standards of known concentration were tested twenty times with one ViBE cartridge.

Sample (ng/mL)	Measured Conc (ng/mL)	SD	CV (%)
50	53.2	5.4	10%
25	24.8	2	8%
5	5.4	0.3	6%

Reproducibility- three samples were tested in duplicate across three lots of cartridges on three different days.

Sample (ng/mL)	SD	CV
44.1	2.5	6%
19.6	1.2	6%
5.2	0.6	12%

Results

To meet today's stringent FDA guidelines with regard to host cell protein detection, biopharmaceutical companies require an assay and sample prep platform that can consistently deliver accuracy, reproducibility, sensitivity and assay performance across a broad dynamic range and with often difficult and complex sample matrices. The BioScale ViBE Platform and AMMP CHO-HCP assay demonstrated performance similar to a validated commercial ELISA but also offered significant advantages with respect to workflow and hands-on time.

Research collaboration supported by EMD Millipore Corporation

Protein A Contamination Assays

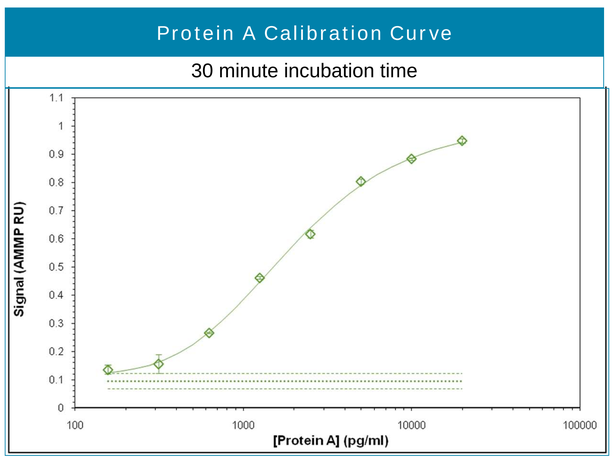
The performance of the ViBE Protein Analysis Platform using the Residual Protein A detection assay was evaluated for feasibility, assay performance and a comparison with a traditional ELISA for Protein A contamination. The data shown a Protein A calibration curve run on the ViBE workstation. Accuracy and precision were evaluated on an assortment of bioprocess samples of a monoclonal antibody selected from multiple stages of the purification process. A commercially available ELISA was run on the same samples for comparison.

Calibration Range

This chart represents a Protein A calibration curve run using the ViBE Workstation and a 30 minute incubation time. The estimated total assay time for 96 assays is 2.5 hours completely unattended.

Protein A Calibration Curve

30 minute incubation time



Assay Performance and Comparative Results

These tables represent data from samples of a Monoclonal antibody produced in Chinese Hamster Ovary cells and using a Protein A purification process. Samples were taken at four stages of a purification process and span the purification process. The first sample is the eluate from the first protein A column. Sample 2 is the load onto the Q column. Sample 3 is the Q column filtrate. Sample 4 is final drug substance. Results demonstrate high quality data integrity for accuracy and precision with low %CVs. The second table shows the reported ELISA results for comparison.

Protein A Assay Accuracy and Precision

	ViBE (pg/mL)	SD	CV	n	ViBE ng/mg
Sample 1 (1:80)	252814	22638	9%	3	14.7
Sample 1 (1:160)	256775	5811	2%	3	14.9
Sample 1 (1:320)	265721	26798	10%	3	15.4
Sample 1 (1:640)	292018	35489	12%	3	17.0
Sample 1 (1:1280)	231815	20084	9%	3	13.5
Sample 2 (1:2)	271	14	5%	4	0.10
Sample 3 (1:2)	311	32	10%	3	0.03
Sample 3 (1:4)	396			1	0.04
Sample 4 (1:4)	2263	72	3%	4	0.03
Sample 4(1:8)	2492	172	7%	4	0.03
Sample 4 (1:16)	2667	117	4%	4	0.03
Sample 4(1:32)	2728	203	7%	3	0.03

Comparative ELISA Assay

	(pg/mL)	ng/mg
Sample 1	206400	12
Sample 2	891	0.33
Sample 3	216	0.02
Sample 4	2367	0.03

Results

The ViBE Workstation using the Protein A Detection Assay generated reproducible results (single-digit CVs) while saving significant FTE time with load-and-go operation. With this same assay, configuration and timing can be adjusted for different speed (incubation time) and sensitivity requirements. Results can be obtained after a 30 minute sample incubation or, with longer incubations, picogram level sensitivity can be achieved with a total assay time for 96 samples of 2.5 hours.

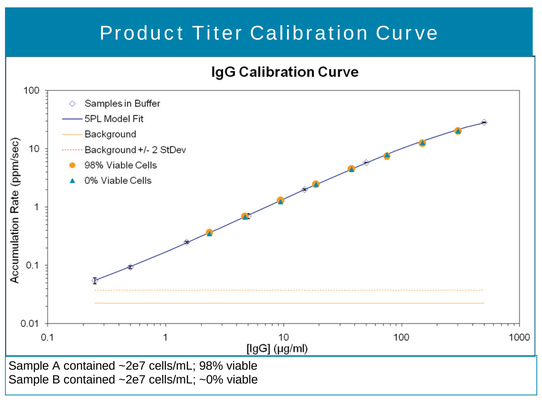
Protein Titer Measurement

Product Titer experiments were run in multiple plates of raw fermentor samples diluted into assay buffer. The samples were not spun before analysis. Calibration curve and testing of samples using viable and non-viable cells were run to assess feasibility. Accuracy and precision was evaluated using three human IgG samples and a correlation study tested samples in duplicate across 8 serial dilutions compared to the commercial reference method.

Calibration Range

The ViBE Workstation successful performed protein measurement with direct Bioreactor samples (**no spin**) regardless of viability. The data in this chart shows spike recoveries in samples with 0% and 98% viable cells that fall in line with calibration.

Product Titer Calibration Curve

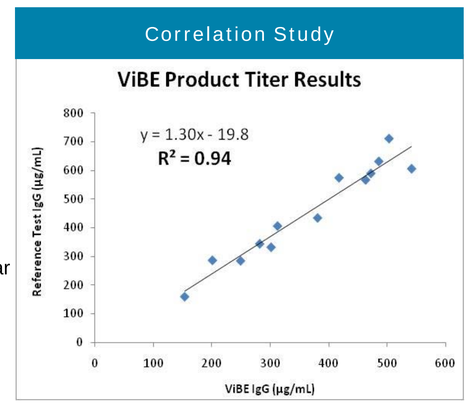


Sample A contained ~2e7 cells/mL; 98% viable
Sample B contained ~2e7 cells/mL; ~0% viable

Comparative Assay Performance

Product Titer results performed on the ViBE Workstation agree well with the commercial reference method (R2 = 0.94). Samples tested in duplicate across 8 serial dilutions, average of linear dilutions was used in the comparison.

Correlation Study



Antibody Titer Accuracy and Precision

Three samples of human IgG were prepared and nine replicates of each were run across the BioScale cartridge accuracy and precision for all three samples met specifications

Antibody Titer

Sample (µg/mL)	Measured Conc. (µg/mL)	Number of Replicates	Standard Deviation	CV	Recovery
400	371	9	10	2.7%	93%
40	42	9	0.6	1.5%	106%
4	4.3	9	0.08	2.0%	107%

Results

The challenges of dealing with complex samples, such as fermentor samples with high cell concentration were assessed with the ViBE Protein Analysis Platform. Using the ViBE, there was no sample treatment required and quantitation was verified with "no spin" samples containing over 10 million cells. Only one dilution was necessary and reproducible results were obtained in 10 minutes.