Comparative HCP Testing Using Traditional and Emerging Technologies

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ABSTRACT

Host cell proteins (HCPs) constitute a major group of impurities for biologic drugs produced in cell culture expression systems. As such, HCPs must be adequately removed and monitored to ensure process consistency and product purity. Broad spectrum enzyme-linked immunosorbant assays (ELISAs) are commonly used to quantitate HCPs, but new technologies may offer advantages over traditional methods.

Six emerging technologies were evaluated for their ability to improve Chinese Hamster Ovary (CHO) HCP monitoring: Perkin Elmer AlphLISA, Meso Scale Discovery (MSD) electrochemiluminescence technology, CisBio Homologous Time Resolved Fluroescence (HTRF), Forte Bio Octet, BioScale Acoustic-Membrane MicroParticle (AMMP) technology, and Gyros Gyrolab immunoassay. Assay precision, accuracy, linearity and range for these platforms were measured and compared to our in-house ELISA platform to evaluate performance. More practical aspects, such as ease of use, reagent costs, and instrument requirements were also compared. All the evaluated new technologies offered both advantages and disadvantages to our current HCP ELISA platform. The results of this evaluation are key in shaping our future HCP monitoring strategy.

INTRODUCTION

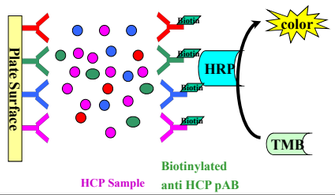
Broad spectrum ligand binding assays are among the methods currently used to detect host cell proteins. These assays are dependent on two critical reagents; 1) a host cell protein reference standard that is representative of the HCPs in the expression system, and 2) polyclonal antibodies directed against those HCPs. Once these reagents are prepared and characterized, they can be used in a number of different technology platforms to detect residual HCP. Traditionally an enzyme linked immunosorbant assay (ELISA) has been used to measure residual HCP. Currently there are many newer technologies that offer enhancements to the traditional ELISA, including Homogeneous Time Resolved Florescence (HRTF), electrochemilumenscence (ECL), Amplified Luminescent Proximity Homogeneous Assay (ALPHA), Octet, Acoustic Membrane MicroParticle (AMMP™) and Gyrolab. While all methods used the same reagent antibodies developed for the traditional ELISA, the reagent antibodies were modified when required following procedures provided by the manufacturer of the technology, without further optimization. Precision, accuracy, linearity, range and sample correlations were used to measure the capability of each platform. Additional observations were made for each of the technologies that included, the number of incubations, the number of washes, time to generate results, additional consumable costs, and instrument costs. All of the data combined demonstrate the similarities and differences for each technology when compared to the traditional ELISA.

Comparison of Platform Parameters

	ELISA	HTRF	ECL	ALPHA	Octet	AMMP	Gyros
Requires an HCP Standard	√	√	√	√	√	√	√
Polyclonal Detection Antibodies	√	√	√	√	√	√	√
Antibody Modification(s)	Unlabeled & Biotin	Cryptate & Acceptor	Biotin & Tag	Biotin & Bead	Unlabeled & Fluoro-phore	Bead & Tag	Biotin & Fluoro-phore
Number of Incubations	4	1	3	3	4	1	1
Number of Washes	3	0	2	0	0	0	0
Range of quantitation (ng HCP/mL)	1-500	5-250	1-10,000	5-400	1-100	8-125	10-10,000
Automation	Yes	No	No	No	No	Yes	Yes
Time to Generate a Result (hours)	8-24	24	24	8	2	18	1.5
Platform Robustness	Good	Good	Good	Fair	Fair	Good	Good
Reagent cost per plate over ELISA	N/A	$20	$100	$225	$ 420	$150	$300
Instrument cost	$50,000	$80,000	$110,000	$80,000	$200,000	$78,000	$250,000

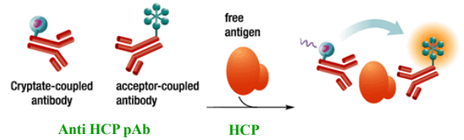
METHOD SCHEMATICS

Traditional ELISA



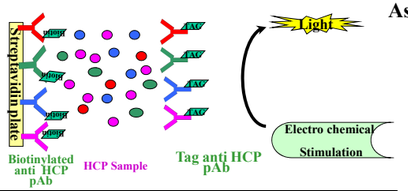
Advantages	Disadvantages
•Industry standard; •extensive experience •Standard microtiter plate spectrophotometer •Can be automated	•Labor intensive, •Requires multiple incubations and washes •Solid state binding

Homogeneous Time Resolved Fluorescence (HTRF)



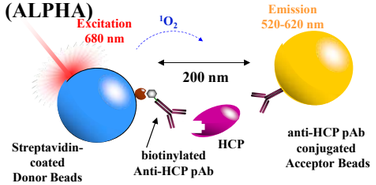
Advantages	Disadvantages
•One step design eliminates washes and incubations •Fluid binding dynamics	•Proximity of the Cryptate and acceptor Abs may be problematic in some samples •No Stability data on conjugated Abs •Requires a high-end fluorimeter

Electrochemiluminescence (ECL)



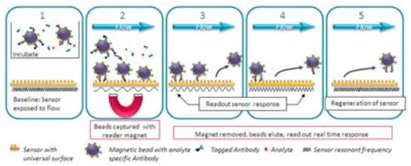
Advantages	Disadvantages
•Method format similar to the ELISA •Fewer washes •Expanded Range of Quantitation •Improved Sensitivity	• Requires multiple incubations • Proprietary micro titer plate •Expensive dedicated instrument

Amplified Luminescent Proximity Homogeneous Assay (ALPHA)



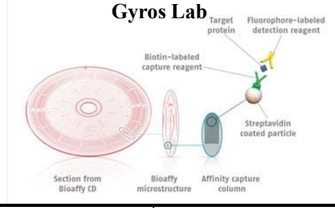
Advantages	Disadvantages
•Sample result generated < 8 hr	•Bead conjugation is variable •Proximity of the acceptor and donor pAbs may be problematic in some samples •Requires low light at certain steps •Purchase and conjugate antibodies to beads •Requires a luminometer

AMMP



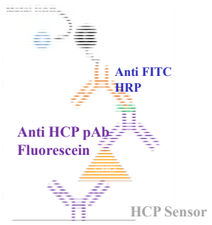
Advantages	Disadvantages
•Walk away •Built in troubleshooting tools	•Requires affinity purified reagents • Requires proprietary flow cell •Requires dedicated instrument

Gyros Lab



Advantages	Disadvantages
•Requires small sample and reagent volumes •Expanded Range of Quantitation •Walk away	•Requires a Bioaffy CD •Requires expensive dedicated instrument

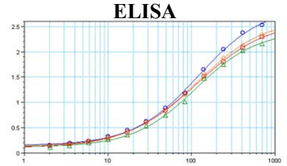
Octet



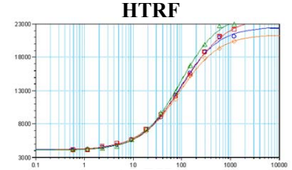
Advantages	Disadvantages
•Good sensitivity •Improved time to first result	•Critical timing of incubation steps • Requires significant hands on time • Instrument limited to one plate analysis at a time • Custom sensors are required

STANDARD CURVE RESPONSES & SAMPLE CORRELATIONS

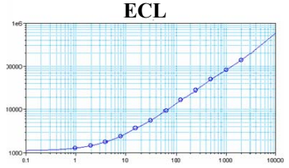
ELISA



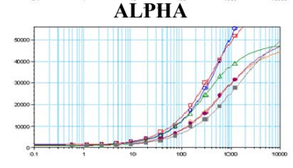
HTRF

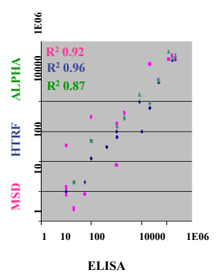


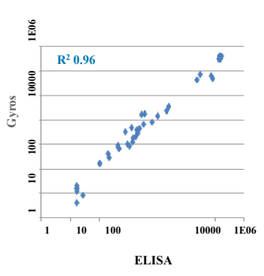
ECL



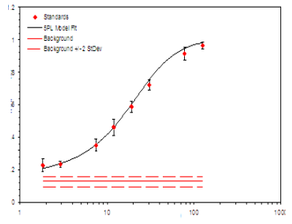
ALPHA



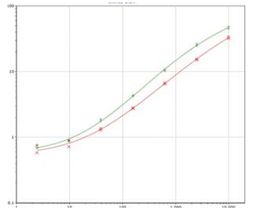


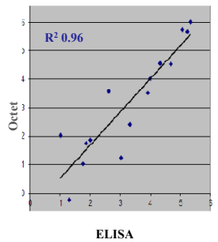


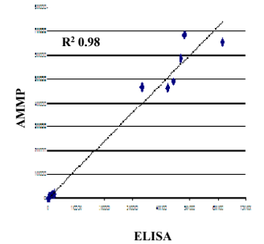
AMMP



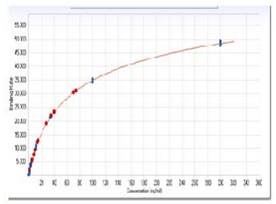
Gyros Lab







Octet



Results & Conclusions

Parameter	ELISA	HTRF	ECL	ALPHA	Octet	AMMP	Gyros
Limit of Detection (ng HCP/mL)	0.3	2	1	ND	0.5	2	1
Range of Quantitation HCP spiked into Assay Diluent (ng/mL)	10-411	5-250	5-10,000	2.5-400	1-100	8-125	10-10,000
Lower Limit of Quantitation HCP Spiked into matrix (ppm)	10	5	5	2.5	1	8	10
Precision (%RSD) Intra- Inter-	<15% (n=10) <20% (n=5)	<20% (n=10) <20% (n=5)	ND <20% (n=5)	< 20% (n=12) <25% (n=5)	ND ≤ 35% (n=4)	≤ 10% (n=6) ≤15% (n=6)	≤ 5% (n=4) ≤ 20% (n=4)
Control Performance (vs ELISA)	Established the range	Within Range	Values out side range (Lower)	Within Range	Within Range	Values out side range (Higher)	Within range
Sample Correlation (vs ELISA)	1	0.96	0.92	0.87	0.96	0.98	0.96

ND: Not determined

- The ELISA continues to provide reliable, precise, and accurate HCP quantitation.
- All evaluated platforms are capable of accurately quantitating HCP in a protein matrix at HCP concentrations ≤ 10 ng HCP per mg of product with ≤ 30%RSD.
- Sample correlations, which ranged from 0.87 to 0.98, demonstrate general agreement between results from each technology and the ELISA.
- The Gyros technology stands out due to its performance characteristics (acceptable precision and lower limit of quantitation; expanded assay range) and efficiency (fully automated, rapid time to first result, reduced sample and reagent consumption), but has high reagent and instrument costs.
- The MSD ECL technology also demonstrated improved performance characteristics (reduced lower limit of quantitation and expanded assay range) and can be automated to increase efficiency.
- The results of this evaluation are key in shaping our future HCP monitoring strategy.