# Host Cell Protein Analysis on the ForteBio Octet Platform

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Bioassay and Impurities Testing

Pfizer, Andover

# Topics

- Introduction to Host Cell Proteins (HCP)
- HCP Analysis
- HCP Method
- ForteBio HCP Application for the Octet
- Results from two Pfizer sites
- Conclusions

# What are Host Cell Proteins?

- **Process related impurities**
  - They are housekeeping proteins that co-express with the protein of choice.
- **Heterogeneous population of proteins that represents the four quadrants of a two dimensional gel**
  - CHO has 10,000's of individual proteins
  - E.coli has 1,000's of individual proteins
- **Host Cell Proteins are not expressed at constant concentrations nor does each species give a similar response in an ELISA**

# Host Cell Proteins Analysis

- HCP analysis is mentioned in ICH guideline 6B
- HCP can be a marker during purification development
- HCP can be a marker to monitor batch consistency, column performance and process validation
- HCP can be a specification for product quality

# Method Development

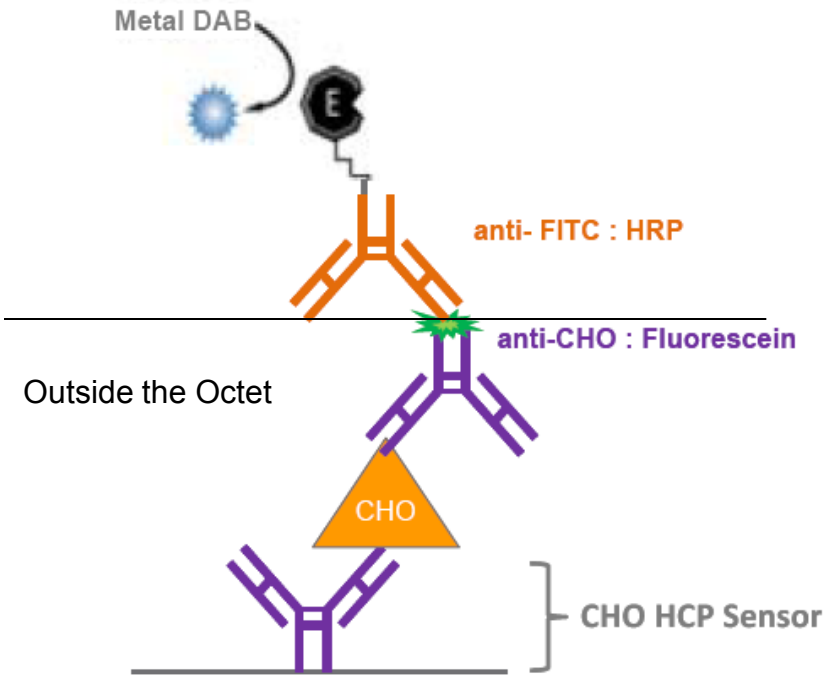
- **Immunological method**
  - Immunogen and standards
    - A set of HCPs collected typically from a null transfected cell line
      - Broad spectrum
      - Enrichment of a subset of HCPs
  - Anti-HCPs
    - Polyclonal
      - IgG cut
      - Affinity Purified against the immunogen or standard
    - Capture reagent
    - Detector reagent
  - Method Output
    - Provide a numeric value for HCP

# Method Comparison

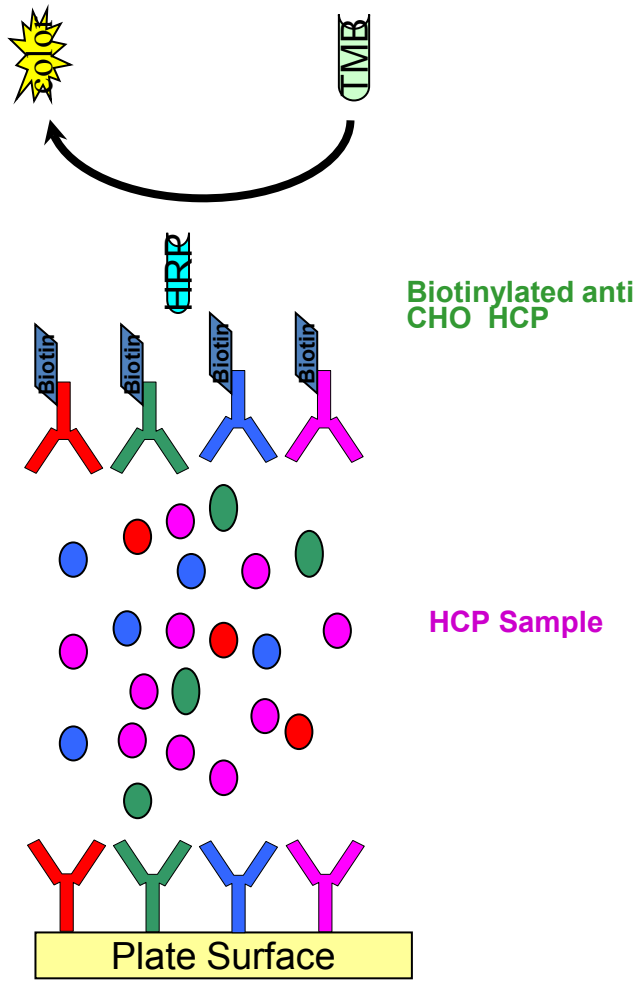
## ForteBio Method

**Assay Protocol:**

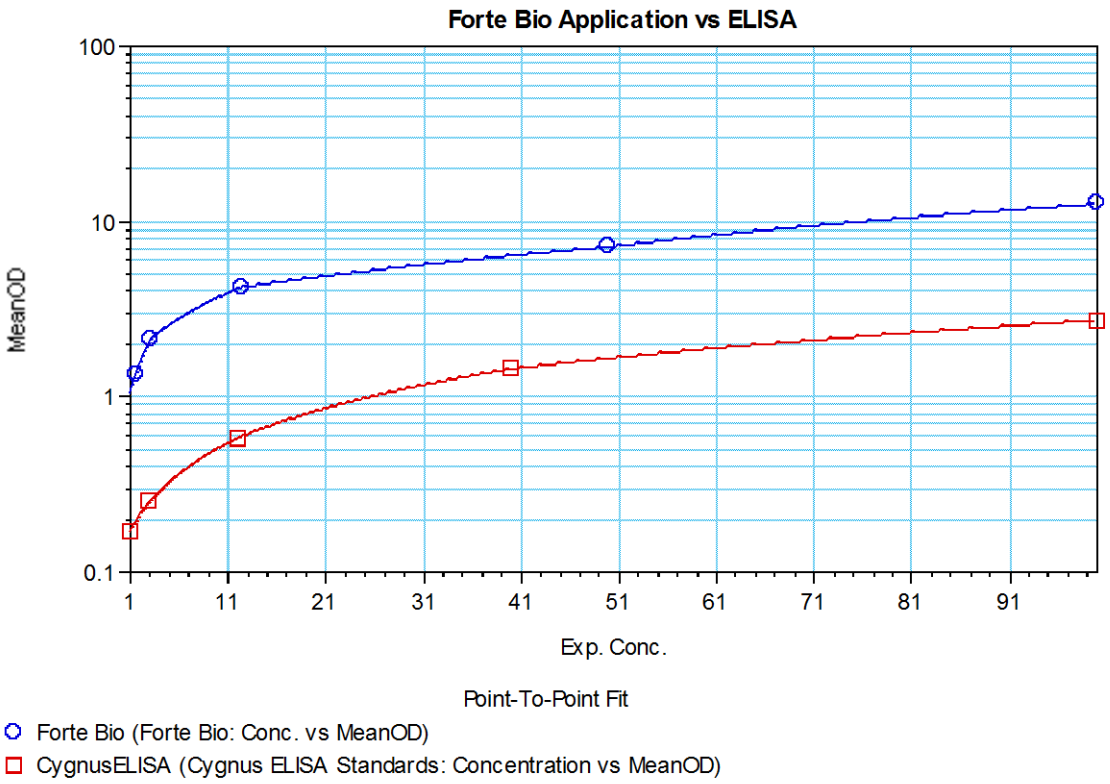
Assay Configuration:




## HCP ELISA



# CHO HCP Standard Curves



# Beta Testing

- **Two-Site Blinded Study**
- **Performance Parameters**
  - Specificity
  - Dilutional Linearity
  - Robustness
  - Accuracy and Recovery
  - Sample Analysis

# Dilutional Linearity

- ForteBio HCP standard was spiked into sample diluent at 500 or 200 ng/mL. The samples were tested in a three fold dilution series for a total of eight dilutions

| Dilution | Dilution Factor | Site 1 HCP (ng/mL) | Site 1 Adjusted Result (ng/mL) | Site 2 HCP (ng/mL) | Site 2 Adjusted Result (ng/mL) |
|---|---|---|---|---|---|
| 1 | 1 | 173 | 173 | 168 | 168 |
| 2 | 3 | 137 | 411 | 80.2 | 241 |
| 3 | 9 | 111 | 999 | 22.9 | 206 |
| 4 | 27 | 29.3 | 791 | 8.16 | 220 |
| 5 | 81 | 11.5 | 931 | 2.93 | 238 |
| 6 | 243 | 4.5 | 1093 | 0.772 | 188 |
| 7 | 729 | 2.02 | 1473 | 0.341 | 248 |
| 8 | 2187 | 0.49 | 1081 | BLOQ | BLOQ |
| Mean | | | 1057 | | 223 |
| SD | | | 257 | | 23.3 |
| CV | | | 24 | | 10 |

Dilutional Linearity is observed starting at 100ng/mL
HCP results are 211% and 112% from expected concentration

# Control Performance

- **Intra assay Precision: Cell Culture Sample Tested in a Dilution Series**

| | Site 1 Ctrl 01 (ng/mL) | Site 1 Ctrl 02 (ng/mL) | Site 1 Ctrl 03 (ng/mL) | Site 1 Ctrl 04 (ng/mL) | Site 2 ELISA (ng/mL) | Forte Bio (ng/mL) | Site 2 ELISA (ng/mL) | Forte Bio (ng/mL) |
|---|---|---|---|---|---|---|---|---|
| | 252 | 83.4 | 35.1 | 14.7 | 21275 | 7199 | 258 | 111 |
| | 211 | 96.5 | 49.9 | 22.7 | 15118 | 7516 | 208 | 98.0 |
| | | 54.1 | 30.9 | 9.22 | 10879 | 5757 | 185 | 84.3 |
| | | 63.4 | 40.1 | 12.2 | 10191 | 6382 | 173 | 68.2 |
| Mean | 232 | 74.4 | 39 | 14.7 | 12063 | 6713 | 206 | 90.3 |
| SD | 28.6 | 19.2 | 8.2 | 5.8 | 2668 | 797 | 37.5 | 18.2 |
| CV | 12.3 | 25.8 | 21.0 | 39.3 | 22.1 | 11.9 | 18.2 | 20.2 |
| Min | 115 | 65.8 | 36.3 | 15.6 | | | | |
| Max | 193 | 104 | 57 | 35.8 | | | | |

**Assay Precision is similar to microtiter plate ELISA's**

# Intermediate Precision

- Two process samples were analyzed with the ForteBio assay on three different days to determine intermediate precision for the assay.

| Sample | Column 1 pool (ng/mL) | Final filter pool (ng/mL) |
|---|---|---|
| **Exp 1** | **6713** | **90.3** |
| **Exp 2** | **9270** | **96.2** |
| **Exp 3** | **15000** | **138** |
| Average | 10328 | 108 |
| Std Dev | 4243 | 26.1 |
| %CV | 41 | 24 |

Intermediate precision for an ELISA is ≤ 10%

# Assay Performance: Recovery and Precision

- HCP spiked into ForteBio sample diluent

| | 0.1ng HCP/mL Site 2 | 1 ng HCP/mL Site 2 | 1 ng HCP/ mL Site 1 | 5 ng HCP/ mL Site 1 | 10 ng HCP/mL Site 2 | 10 ng HCP/ mL Site 1 |
|---|---|---|---|---|---|---|
| | 0.321 | 0.982 | 0.894 | 4.11 | 11.5 | 7.66 |
| | BLOQ | 1.05 | 0.850 | 5.15 | 13.1 | 12 |
| | BLOQ | 0.911 | 0.883 | 4.13 | 11.0 | 13.5 |
| | 0.330 | 0.985 | 0.841 | 4.95 | 10.8 | 12.8 |
| | BLOQ | 1.01 | 0.761 | 5.48 | 12.0 | 11.3 |
| | BLOQ | 0.896 | 0.749 | 5.07 | 10.2 | 10.7 |
| | BLOQ | 0.851 | 0.894 | 6.29 | 10.4 | 15.1 |
| | 0.376 | 0.835 | 0.806 | 3.83 | 9.72 | 8.83 |
| Mean | | 0.94 | 0.83 | 4.88 | 11.1 | 11.5 |
| SD | | 0.8 | 0.06 | 0.82 | 1.1 | 2.43 |
| CV | | 8.3 | 6.9 | 16.8 | 9.8 | 21.2 |
| % Recovery | | 94.0 | 83.5 | 97.5 | 111 | 115 |

% Recovery and precision at low concentrations of HCP is acceptable

# Assay Performance: Accuracy

- HCP spiked into 1mg/mL Ab matrix

| | 1 ng HCP/ mg Ab 1ppm | 2 ng HCP/ mg Ab 2ppm | 5 ng HCP/ mg Ab 5ppm | 10 ng HCP/ mg Ab 10ppm |
|---|---|---|---|---|
| | 0.757 | 1.84 | 4.37 | 11.6 |
| | 0.711 | 1.57 | 4.17 | 10.8 |
| | 0.809 | 1.63 | 6.63 | 11.2 |
| | 0.739 | 1.64 | 3.91 | 10.2 |
| Mean | 0.754 | 1.67 | 4.77 | 11.0 |
| SD | 0.04 | 0.12 | 1.25 | 0.6 |
| CV | 5.5 | 7.0 | 26.3 | 5.5 |
| % Accuracy | 75.4 | 83.5 | 95.4 | 109.5 |

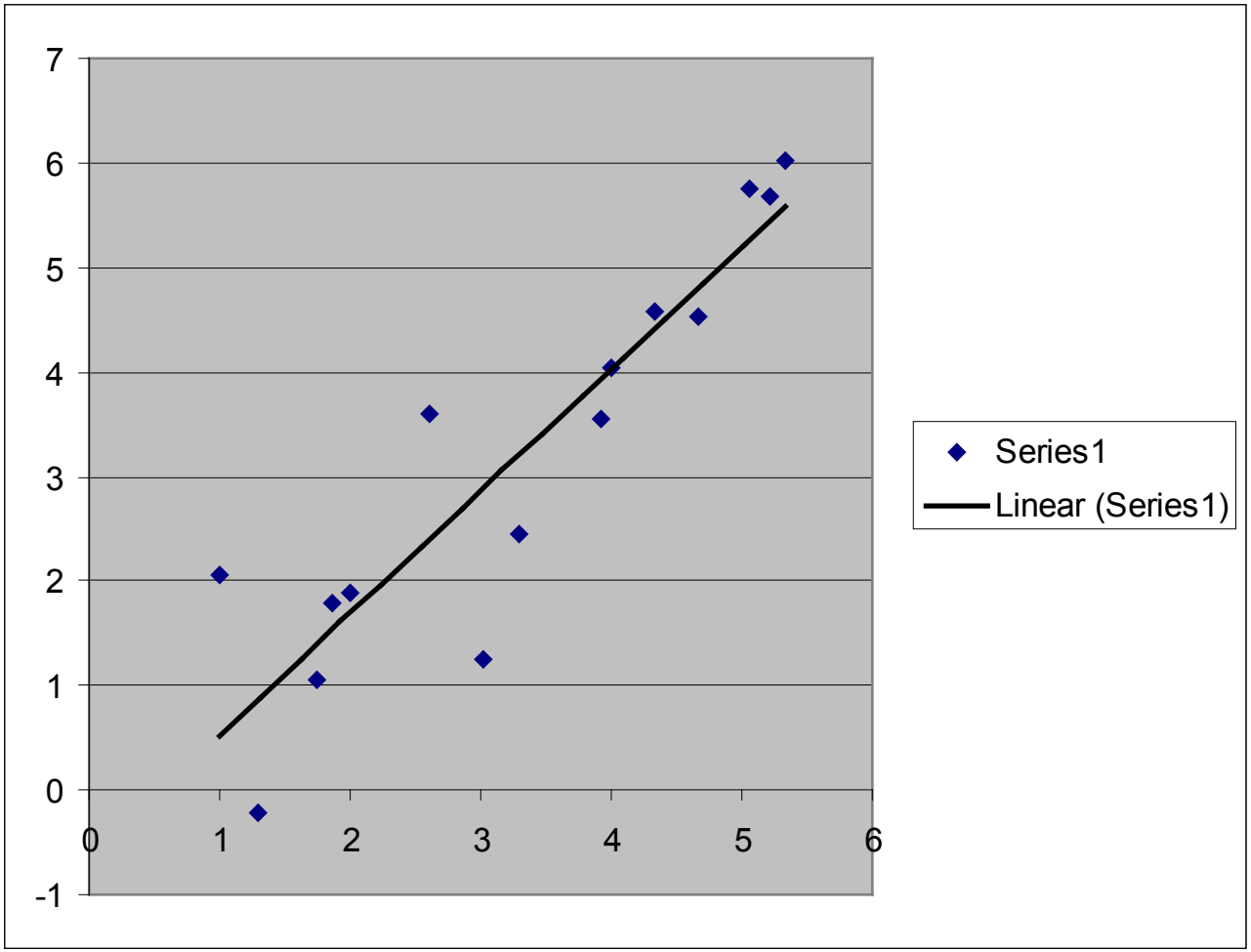
% Accuracy was demonstrated at 1ng HCP/mg Ab matrix (1ppm)

# Sample Performance Site 1

- Three antibodies and two proteins were monitored in the ForteBio HCP Assay

| Sample Description | Site 1ELISA Results (ng/mL) | ForteBio Results (ng/mL) |
|---|---|---|
| Ab 1 Harvest | 114860 | 562000 |
| Ab 1 Column 1 Load | 215209 | 1048000 |
| Ab 1 Column 2 Load | 1046 | 18.3 |
| Ab 1 Column 2 Peak | <20 | 0.612 |
| Ab1 Final Pool | <10 | 114 |
| Protein 1 Column 2 Load | 46921 | 34280 |
| Protein 1 Column 3 Load | 21074 | 37000 |
| Protein 1 Column 3 Peak | 8205 | 3496 |
| Protein 1 Column 3 Wash | 100 | 76 |
| Ab 2 Column 2 Load | 72 | 60 |
| Ab 2 Column 2 Peak | 56 | 11 |
| Ab 2 Column 2 Final Pool | 10046 | 10900 |
| Ab 3 Final Pool | 414 | 4000 |
| Protein 2 Column 1 Load | 166411 | 472500 |
| Protein 2 Column 2 Load | 2013 | 280 |
| $R^2$ | | 0.96 |
| Slope | | 4.27 |
| Intercept | | -21992 |

**HCP results of the 15 samples plotted on a log, log scale**



# Sample Performance

- ForteBio HCP Analysis Across Purification Steps

| Protein | | | | |
|---|---|---|---|---|
| Step | Batch 1 ng/mL | Fold Reduction | Batch 2 ng/mL | Fold Reduction |
| Cell Culture | 525000 | | 1739500 | |
| Column 1 Load | 320400 | | 1583100 | |
| Column 1 Peak | 8.43 | 38007 | 49.5 | 31981 |
| Column 2 Peak | 11.4 | 0.74 | 302.4 | 0.16 |
| Antibody | | | | |
| Column 1 Load | 305500 | | | |
| Column 1 Peak | 129500 | 2.4 | | |
| Column 2 Peak | 1918 | 67.5 | | |
| Final Pool | 2639 | 0.7 | | |

# Comparison of ForteBio and ELISA for HCP Clearance Site 2

| | CHO HCP ForteBio Assay | | PS CHO HCP ELISA | |
|---|---|---|---|---|
| mAb 1 | HCP (ng/mg) | Fold Removal | HCP (ng/mg) | Fold Removal |
| Harvest | 68163 | | 95476 | |
| Column 1 Pool | 744 | 92 | 599 | 159 |
| Column 2 Pool | 96.2 | 7.7 | 61.8 | 10 |
| Column 3 Pool | 21.6 | 4.5 | 47.9 | 1.3 |
| Final Filter Pool | 19.7 | 1.1 | 29.3 | 1.6 |
| mAb 2 | | | | |
| Column 1 Pool | 300 | | 169 | |
| Column 2 Load | 40.2 | 7.4 | 18.4 | 9.2 |
| Column 3 Load | 32.9 | 1.2 | 14.6 | 1.3 |
| Final Filter Pool | 1.0 | 33 | 1.1 | 14 |
| mAb 3 | | | | |
| Column 1 Load | 258,611 | | 229,326 | |
| Column 2 Load | 475 | 544 | 287 | 800 |
| Column 3 Load | 109 | 4.4 | 33.5 | 8.6 |
| Column 3 Eluate | 10.1 | 11 | 10.2 | 3.3 |

# Conclusions

- The ForteBio HCP assay range is 1 to 100 ng HCP/mL
- Dilutional Linearity is observed from 0.4 to 100 ng HCP/mL
- Recovery was demonstrated at 1ng HCP/mL
- Several of the assay parameters are equivalent to established HCP ELISA
- The specificity of the reagents demonstrated adequate HCP removal across purification processes
- This application would be applicable for measuring HCP in down stream samples
- The ForteBio HCP application offers a sample turn around time of 90 minutes for 7 samples tested as duplicates or 14 samples tested in single wells

# Thanks

- **ForteBio**
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