Workshop Review\(^{(1)}\): Host Cell Protein Impurity Analysis
Dubrovnik, Croatia
May 15-16, 2014

\(^{(1)}\) Illustrated with Genentech data
Increasing interest in Host Cell Protein analysis

- Workshop in Lisbon, associated with BEBPA BioAssay conf., 2012
- Joint BEBPA-USP conference, Washington DC, June 2013
- BEBPA Workshop, Dubrovnik, May 2014
- WCBP Workshop, Washington DC, January 27-29, 2015 (CaSSS.org)
- Planned BEBPA West Coast USA Workshop for May 2015

USP Guidance Document due out early 2015
  (Maura Kibbey, MCK@USP.org)

<1132> Residual Host Cell Protein Measurement in Biopharmaceuticals

EuP Guidance Document being drafted
FDA emphasis on Quality

The Biopharmaceutical industry needs to produce products with low levels of impurities.

Health Authorities and patients expect nothing less.

FDA concerns about HCPs
- Immunogenicity
- Biological activity

Frequently asking questions about the suitability of your HCP control system.
~24,383 predicted gene products

Broad range of proteins recognized by anti-CHOP antibodies

- Proteins not equally recognized
- Abundant vs rare/absent antibodies
- Single number “result” for HCP assay is “immunologically weighted”

The issue is one of demonstrating coverage

Why do some HCPs co-purity with product?

- CHO protein impurity levels depend on the particular mAbs
- “hitchhiker” effect – interaction of product and CHOP

Antigen Excess Can Result in dilution dependence of the assay

Product Concentration

<table>
<thead>
<tr>
<th>Concentration</th>
<th>CHOP-A</th>
<th>CHOP-B</th>
</tr>
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<tbody>
<tr>
<td>10 mg/ml</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>5 mg/ml</td>
<td>*</td>
<td>*</td>
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<tr>
<td>1 mg/ml</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>0.5 mg/ml</td>
<td>*</td>
<td>*</td>
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<tr>
<td>0.3 mg/ml</td>
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</table>

Non-linear response indicates potential host cell protein impurity in excess of the available antibodies.

Chromatography used to Separate HCPs from product

Separation of product from CHOP using ceramic hydroxyapatite (CHT)

CHOP

Product

MS used to identify predominant HCP(s)

Identification of Phospholipase B-like 2 (PLBL2) a CHOP impurity in antigen excess

Procedure:

1) SDS-PAGE of pooled ELISA positive fractions from HPLC

2) Excise and digest bands resolved on gel

3) Analyzed with nano LC-MS/MS

4) Searched mammalian UniProt sequence database

5) Confirmed the sequences by MS and MS2

Coverage Report for Phospholipase B-like 2 (PLBL2) protein

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  1 MAAPDMRDSPG GRARVRAALR A LALASLTTEVL LNCPAGALPT QGPGRRQLNL
  51 DPPWRSRVS LLLDAASGQLR LDVGIHPPAV AWANLNTAIR ETGWAYDLG
 101 TNGSYSNDLQ AYAAGYVEAS VSEEILYMHW MNTVNYCGRF FEYEVGYCEK
 151 LKSFLEINLE WMQREMELSQ DSPYWHQVRLL TLLQLKGLED SYEGRLTEPT
 201 GRTIKPLGF LLLQIAGDLE DLEQALNTKS TKLGLGSGSC SAIILGPAG
 251 RDLVAHNTW NSYQNMLRIKT YQQLQFRQG PQEATPIAGNNLVSSYPG
 301 TlSFGDFFYIGGSGLVTEL TIGNKNPALW KVQPGQQCVLEWIRNINARN
 351 LALDGATWD IFKQFNSGTY NNQWMTFDYK AFIPNGPSPG SRLTILEQI
 401 PGNHVVDKT EDLYKTTYWA SYNIPFEEILFVNASGLQQLV AQYCDWSYFT
 451 KNPRQIFGQR DQSLVedmns MVRIRYNNF LHDPLSLCEA CIPKPNENA
 501 I SARSDLNA P A NGSYFQALY QRPQGIDVK VTSFSLAKRM SMLAASGPTW
 551 QLPFPQWSSL SPFRTSMLMG QPDLWTFSPI SVRWD
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Key

| Minimum PSMs | 0 | 1 | 2 | 5 | Modified |

Coverage: 195 / 585 (33%)
A few more details on PLBL2

• PLBL2 gene cloned, expressed in CHO cells, purified, used as immunogen and analytical standard. PLBL2-specific sandwich ELISAs developed based on rabbit polyclonal and mouse monoclonal antibodies developed in-house.

• Mammalian PLBL2 is much different from non-mammalian Phospholipases.


• Hamster and human PLBL2 are about 20% different in amino acid sequence, with many of the differences in surface residues – likely to be immunogenic.

• PLBL2 can be reduced to <1 ng/mg with good purification processes. All Genentech and Roche antibodies now screened for PLBL2 and purification improvements implemented where needed.

• Our platform CHOP ELISA detects PLBL2, however other CHOP ELISAs have been found that do not detect PLBL2.

Vanderlaan et al. (in preparation) Hamster Phospholipase B-Like 2 (PLBL2), a host cell protein impurity in CHO-derived therapeutic monoclonal antibodies.