Background to the General Chapter

• 2011 EDQM Workshop on the future of monographs in the biological field

• Identify assessors’ needs and expectations
  ➔ Need for the Ph. Eur. to tackle host-cell protein assays
  ➔ Address questions that are often posed during assessment, e.g. use of commercial kit vs product-specific assay, development and validation of a process-specific assay
Types of HCP assay

**Process-specific assays (also called product-specific HCP assays)**

- Developed and validated taking into account the specificity of the production process and using the host organism expressing the recombinant product

- Antigen derived from a mock run of the drug substance manufacturing process (or a process representative of it) up to a step capable of generating sufficient quantities and broad spectrum of HCP

- Antisera raised need to have a broad coverage of HCPs, to be able to detect as many different HCPs as possible and thereby also accommodate process variations
Types of HCP assay (cont’d)

Platform assays
- Developed by individual manufacturers and customised for their expression host and processes
- Same sets of reference standards and reagents may be used to monitor HCPs in several products manufactured in the same expression host, provided that upstream (and downstream, if relevant) processes are sufficiently similar between these products

Types of HCP assay (cont’d)

Generic assays
- Commercially available HCP test kits are commonly referred to as generic HCP assays
- They are intended to work broadly across similar expression hosts
- Detailed information on the preparation of the assay may not be disclosed by the vendor. For instance, the null cell line may be derived from a combination of strains of an expression host species, and the process(es) used may not mimic the process applied for the product of interest.
## Production and Testing of the HCP Antigen

<table>
<thead>
<tr>
<th>Process specific assays</th>
<th>Platform assays</th>
<th>Generic assays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Null cell line</strong></td>
<td>- Derived from the same cell line</td>
<td>- Same host organism across a company’s portfolio</td>
</tr>
<tr>
<td><strong>Mock manufacturing process – Upstream</strong></td>
<td>- Mimics the intended process - May be adjusted to cover worst case situations</td>
<td>- Mimics the platform upstream process that is used for several products</td>
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<tr>
<td><strong>Mock manufacturing process – Downstream</strong></td>
<td>- Minimal processing recommended - Further processing could be considered</td>
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<tr>
<td><strong>Characterisation and testing</strong></td>
<td>- Comparison from pools collected at relevant stages of the mock vs intended process</td>
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## Production and characterisation of the anti-HCP antibody reagent

<table>
<thead>
<tr>
<th>Process specific &amp; Platform assays</th>
<th>Generic assays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunisation</strong></td>
<td>- Animal species: host that yields sufficient amounts and diversity of HCP-specific IgG - Aim: immune response against both strong and weak antigens</td>
</tr>
<tr>
<td><strong>Purification and preparation</strong></td>
<td>- Protein A- or protein G- chromatography and/or HCP antigen affinity chromatography - Removal of aggregates may be required</td>
</tr>
<tr>
<td><strong>Characterisation and testing</strong></td>
<td>- <strong>Demonstration of coverage</strong>: comparison immunostain vs total protein stain by 2D electrophoresis</td>
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Validation of HCP assay

- *Accuracy, Specificity, Precision, Range and Robustness*
  - As for any other quantitative assay
- *Quantitation and detection limits*
  - Sensitivity: ppm range
  - Quantitation limit determined (HCP spike recovery studies); Detection limit often not determined
- *Linearity*
  - Linearity of the assay (dilution series of the HCP standard, spike recovery experiments)
  - Sample *dilution non-linearity* to be assessed:
    - Comparison of target versus measured HCP concentration at varying sample dilutions
    - Dilution linearity is demonstrated if the acceptance criteria for assay variation are met for the differently diluted samples
  - Reporting of HCP value: results obtained for at least two dilutions within the linear dilution range are averaged

Change of HCP assay and/or HCP assay reagent

- Consider re-characterisation and re-validation of the assay:
  - **Generic assay**: for each new batch of reagent
  - **Process specific assays and platform assays**:
    - When HCP reference standard and / or antibody are depleted
    - Following a process change that can impact the HCP composition
- Recommendations for reagent characterisation and assessment of the validation status of the assay
European Pharmacopoeia Commission
Decision on work program

Group of expert or Working party
Preparation of draft document

Public consultation on Pharmeuropa 27.2 (April 2015)
(Comments by 30 June 2015)

Group of expert or Working party
Examination of comments

European Pharmacopoeia Commission – 154th session
Adoption (March 2016)

Supplement 9.1 (Publication: October 2016)
Effective 1 April 2017

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- HCP Working Party Members