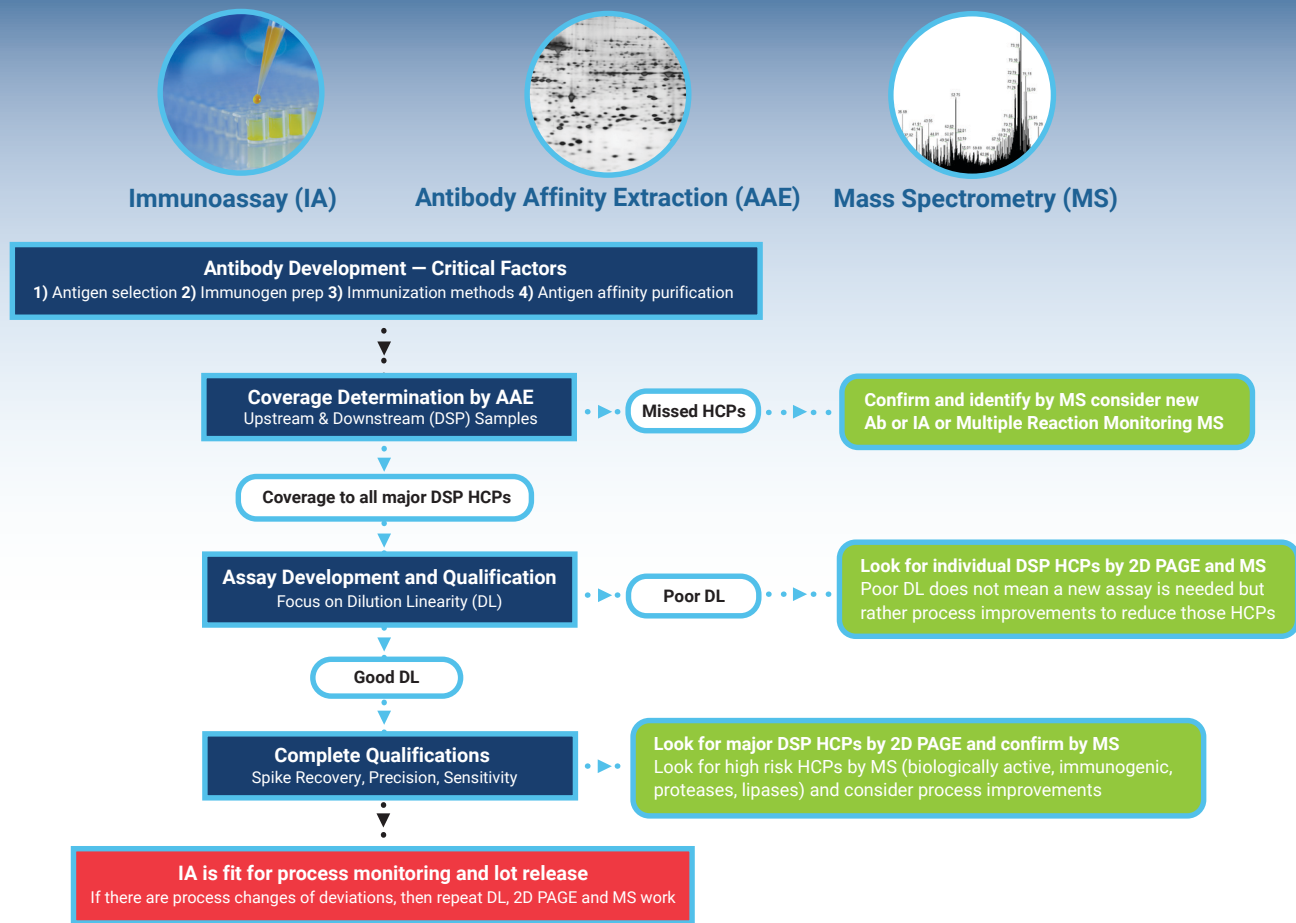# Integration of Advanced Orthogonal Methods for HCP Characterization

**Step 1:** HCP antibody and ELISA development using proper upstream source of antigen

**Step 2:** HCP antibody coverage analysis using AAE followed by 2D-PAGE or MS

**Step 3:** ELISA qualification by spike recovery, precision, and sensitivity studies

**Immunoassay (IA)**    **Antibody Affinity Extraction (AAE)**    **Mass Spectrometry (MS)**

**Antibody Development — Critical Factors**
**1)** Antigen selection **2)** Immunogen prep **3)** Immunization methods **4)** Antigen affinity purification

**Coverage Determination by AAE**
Upstream & Downstream (DSP) Samples

**Missed HCPs** → Confirm and identify by MS consider new Ab or IA or Multiple Reaction Monitoring MS

**Coverage to all major DSP HCPs**

**Assay Development and Qualification**
Focus on Dilution Linearity (DL)

**Poor DL** → Look for individual DSP HCPs by 2D PAGE and MS. Poor DL does not mean a new assay is needed but rather process improvements to reduce those HCPs

**Good DL**

**Complete Qualifications**
Spike Recovery, Precision, Sensitivity

→ Look for major DSP HCPs by 2D PAGE and confirm by MS. Look for high risk HCPs by MS (biologically active, immunogenic, proteases, lipases) and consider process improvements

**IA is fit for process monitoring and lot release**
If there are process changes of deviations, then repeat DL, 2D PAGE and MS work

**Step 4:** Mass Spectrometry

✓ AAE enables removal of DS interference and enrichment of HCPs. While HCPs can be identified and quantified by discovery and targeted MS, mass spectrometry will fail to detect lower abundance HCPs without their enrichment.

✓ In the cases where there is a lack of dilution linearity by ELISA, or individual HCPs, these HCPs should be identified and quantified by mass spectrometry.

✓ If HCPs are identified by MS and are not being accurately quantified by the total HCP ELISA, then consider development of a mono-specific ELISA or a targeted MS method, or optimize the purification process to better remove HCPs.

✓ If there are no detectable individual HCPs in a DS by 2D PAGE, the ELISA shows low total HCP that is confirmed by a "reference" ELISA, and if downstream and DS samples show dilution linearity, then MS serves as a confirmatory orthogonal method.

Contact us at **techsupport@cygnustechnologies.com** to discuss your current and future HCP analysis projects!

## Trust Your Assays. Trust Your Results.

## cygnustechnologies.com

**CYGNUS**
**T E C H N O L O G I E S**
part of **Maravai LifeSciences**