The technical and methodological challenges of a successful Host Cell Protein (HCP) ELISA development are manifold. The selection of a suitable antibody for the immunodetection of a given HCP mixture is crucial. First of all, an important decision must be made regarding the use of affinity-purified anti-HCP antibodies versus the use of a Protein A-purified total IgG fraction. Furthermore, the authorities request a sufficient coverage of the selected antibody, which is usually determined by 2D electrophoresis/Western Blot. The E. coli case studies presented here address two different aspects: (1) We discuss the common advantages and disadvantages of using affinity-purified antibody and its corresponding total IgG fraction for HCP ELISA measurement of process samples. (2) We discuss the impact of the technical equipment on HCP coverage determination using 2D electrophoresis/Western Blot.

Antibody Testing by 1D Western Blotting
The performance of both antibody preparations was qualitatively analyzed by 1D Western blotting. The respective process-related mock HCP, as well as a panel of process samples, was subjected to electrophoresis and subsequently transferred to nitrocellulose membranes. The total protein pattern was visualized using reversible Ponceau 5 staining (Figure 1) prior to HCP-specific immunostaining (Figures 2 to 4). The staining results of the different antibodies and the unpurified antisera pool were compared. Detection was enabled using a suitable anti-Rabbit IgG PerkinElmer conjugate followed by incubation with a colorimetric substrate. Significantly different immunostaining patterns, particularly at low molecular weights (approx. <58 kDa), were observed for the process-related mock HCP and the early process sample when comparing the unpurified antisera and the derived antibodies (Figures 2 to 4, lanes 5 and 6). While the total IgG mimicked coverage performance of the unpurified antisera pool well, immunostaining with the specific IgG revealed fewer and less intense signals in the low molecular weight region (highlighted in Figures 1 to 4).

Impact of 2D Electrophoresis Instrumentation on Sample Resolution
Coverage determination by means of 2D Western blotting is widely performed for proving HCP antibody suitability for a specific process. Technological progress has been achieved regarding 2D electrophoresis instrumentation by setting up horizontal flatbed electrophoresis for large 2D gels. Spot resolution of complex HCP mixtures greatly improves when applying High Performance Electrophoresis (HPE), when compared to traditional electrophoresis systems, which invoke a vertical setup for the second dimension. In particular, low molecular weight proteins are visualized well using HPE and take part in subsequent analyses (Figure 5).

Sample Resolution Impacts 2D Western Blot Based Coverage Values
Up-to-date 2D electrophoresis instrumentation enables more detailed visualization of sample HCPs including the low molecular weight (LMW) proteins. Naturally, LMW proteins are less immunogenic than larger protein species, and tend to be difficult to detect simultaneously on the same 2D western blot. There is an increased challenge of achieving sufficient coverage of the highly diverse HCP population, which differs not only in abundance but also in immunogenicity. Consequently, 2D Western blot based coverage values tend to be lower compared to past years. The case study presented here demonstrates how the representation of the same E. coli derived HCP mock sample changes upon improving the secondary dimension. Anti-HCP antibody coverage consequently dropped from 89% to 69% (Figure 2).

Conclusion
Even though 2D Western blot analysis indicated a loss of certain antibody species, during antigen-specific affinity purification, we recommend selecting the specific IgG for final ELISA development in the following three points:

> HCP ELISA is the default system for drug substance batch release testing.
> Highly similar HCP impurity levels were measured by ELISA in the crucial bulk drug substance indicating loss of only ‘irrelevant’ antibodies species during affinity purification.
> The use of specific IgG enabled clearly higher assay sensitivity which is crucial for reliable and robust measurement of HCP trace amounts.

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