TotalLab
LIFE SCIENCE ANALYSIS

The TotalHCP Project

The Problem

Host Cell Protein analysis is fragmented, with no single technique providing enough information to fully characterise the host cell proteome. Three different techniques, at least two are recommended, are required to compensate for their respective limitations and fully characterise the HCP profile of advanced therapeutics.

Anti-HCP ELISA is the current gold standard for monitoring HCP clearance during process development and for final product release. It enables robust and high-throughput quantification of residual HCP levels. However, ELISA does not allow simultaneous quantification of Total HCP content and identification of HCPs. To combat these limitations, 2D SDS-PAGE and Western blotting is recommended to validate the anti-HCP ELISA antibodies by providing high-resolution separation of HCP impurities and approximate MW and PI which can help with identification.

Absolute Identification of specific HCPs has rapidly become the domain of mass spectrometry. However, mass spectrometry is not without its own significant limitations:

- Mass spectrometry can only identify HCPs present within the comparison database. Recent research from last year's BEBPA conference identified significant differences in identification between databases even within the same host cell species.
- Mass spectrometry faces major challenges in terms of sensitivity and quantitation of sufficiently large sets of heterogeneous HCPs.
- QC-related issues when it comes to quantification. In part, this is believed to be because laboratories are repurposing systems intended for proteomics use for HCP analysis.

The Proposed Solution

The TotalHCP Project aims to tackle these issues using software, to simplify and improve the process of HCP clearance validation. The first step will be combining the outputs of different technologies into one easy-to-use dashboard, allowing that data to be easily submitted in one complete report. Next will be the creation of mass spectrometry-specific software tailored to the needs of HCP analysis.

Goals for the mass spectrometry software include:

1. Verifiably correct identification of low abundance Host Cell Proteins.
2. Workflows to support calibration for improved quantitative analysis, setup of experiments and reporting.
3. Built-in QC checks to warn users of issues like changes to global settings on the mass spectrometer.
4. Support for internal and cross-industry libraries to identify and distinguish Host Cell Proteins from the product and laboratory/manufacture-introduced contaminants.
5. Automation to lessen the manual work needed to perform an analysis and reduce the potential for mistakes and inter-operator variability.

Anti-HCP ELISA

- Total HCP Content
- Total HCP Quantification
- Individual HCP Identification

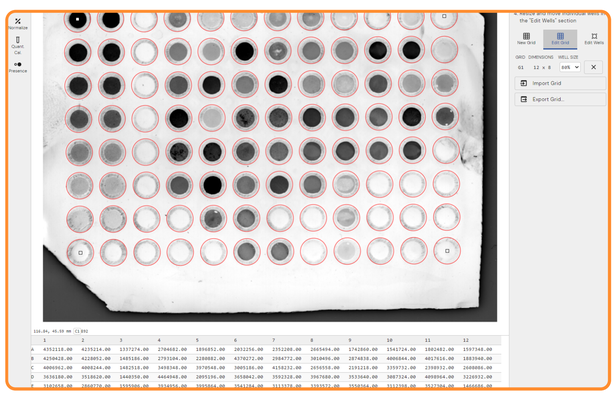
Mass Spectrometry

- Individual HCP Identification
- HCP Visualisation
- Limited Quantification

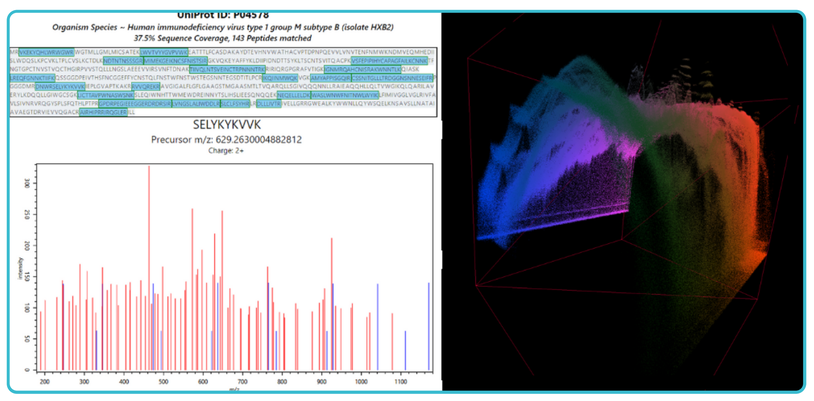
2D SDS-PAGE + WB

- Individual HCP Identification
- HCP Visualisation
- Limited Quantification

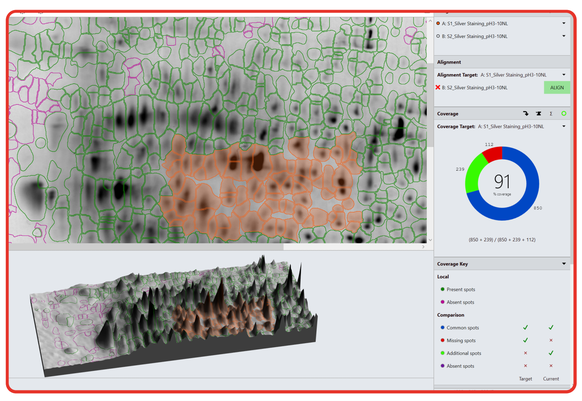
Phoretix Array



SpotMapMS



SpotMap



Total HCP Characterisation

How You Can Help

We cannot deliver on these goals without help from the current experts in the field. We are therefore looking to engage with interested parties at the BEBPA 2024 conference.

The TotalHCP project offers those working within the field of HCP analysis a unique opportunity to work with a team of software developers to guide the development of a purpose-built piece of software, and to make sure it does exactly what you need it to do. This work will build on work already ongoing with the Royce Institute in Manchester, who have designed and are acquiring a 200 sample mass spectrometry DIA dataset on 5 different machines to enable us to take a more rigorous, scientific approach in ensuring that our software can really deliver the best possible results for HCP analysis.

To get in contact to discuss how you can help influence the next generation of HCP analysis software, please contact will.dracup@totallab.com

UKRI Innovate UK
HENRY ROYCE INSTITUTE
MANCHESTER 1824 The University of Manchester