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Poster #95: Universal host contaminant protein identification and quantification for any production host by LC-MS/MS

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Objective
Both the Eu. Ph. Chapter 5.13 and the EMA concept paper on manufacturing of phage products demand controlling host-cell derived impurities, e.g. host-cell proteins (HCPs), endo- and exotoxins, as well as prophages. Here, we present a universal method for HCP control of phage products by LC-MS/MS.

Methods
E. coli phage T7 was spiked with EcoCAT protein standards and measured via untargeted LC-MS/MS. HCPs were detected and quantified a) relative to the phage proteins, b) relative to the spiked standard, and c) relative to an E. coli lysate.

Results
In a semi-purified T7 sample, we detected over 1,250 E. coli host proteins. 13 HCPs were directly quantified by using the internal EcoCAT standards. By correlation of all HCP signals to these 13 datapoints, we estimated the absolute quantification of all 1,250 E. coli host proteins and calculated the total HCP protein abundance.

Conclusion
LC-MS/MS-based HCP testing enables highly sensitive and specific HCP detection independent of the host strain or organism. By using internal or external standards, total HCP levels can be calculated similar to the results of an ELISA. Additionally, LC-MS/MS based methods deliver quantitative results on single protein level. These additional data facilitate method development and risk assessment for therapeutic phages.

Reference #1

Reference #2

Reference #3

Author: SIGLOCH, Florian (PolyQuant GmbH)

Co-authors: BATSCH, Michelle (PolyQuant GmbH); Dr WELZ, Tobias (PolyQuant GmbH); Dr FORCHHEIM, Michael (PolyQuant GmbH)

Presenter: SIGLOCH, Florian (PolyQuant GmbH)

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