

Gyrolab® technology provides robust, operator-independent host cell protein (HCP) detection with high throughput across global development sites

CASE STUDY

- This case study shows an automated Gyrolab immunoassay setup that enabled reproducible results independently of operator and biologics development site
- The study demonstrates high-throughput host-cell protein (HCP) detection in samples from different purification stages using the Gyrolab system linked to a liquid handling robot
- Detection of HCP impurities in biologics and viral vector therapeutics production is essential both from a patient safety and regulatory perspective



Case study: Gyrolab technology provides robust, operator-independent HCP detection with high throughput across three global biologics development sites

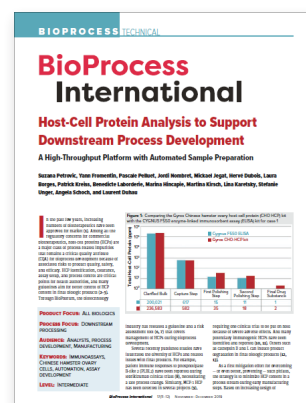
Cell line expression of biologics or viral vectors may leave behind host cell protein (HCP) impurities in the final product that can cause unwanted immune response. HCP detection is therefore essential in biologics manufacturing and assays need to be run frequently without interruptions. HCPs are typically measured by enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies targeting the host cell line but the throughput and variability of ELISAs may not meet the requirements of the development teams.

In a publication Petrovic *et al* demonstrate the reliable high-throughput detection of HCP contaminants using the Gyrolab platform. They also confirm that the results were highly consistent across three biologics development sites for all tested projects and related process steps. This showed the method to be operator- and site-independent, enabling transfer of projects across sites.

Petrovic *et al* BioProcess International (2019). [Download article](#).

Background

Protein development teams are dependent on reliable HCP analysis methods when optimizing conditions for each process step. In order to manage the increasing numbers of samples they need a high-throughput solution while retaining the quality achieved with the HCP ELISAs typically used. The Gyrolab platform is widely used for pharmacokinetic (PK) and toxicokinetic (TK) analyses, and increasingly used for detection of impurities such as HCPs. The robustness of the Gyrolab platform for high throughput immunoassay analysis, also in regulated environments, made the authors turn to this method to address the increased demand for sample throughput to support the production of antibodies and therapeutic proteins.



Results

Good agreement between Gyrolab CHO HCP assays and manual (reference) ELISA

In order to benchmark the performance of the Gyrolab technology for running Chinese hamster ovary (CHO) cell HCP analysis, the authors did a side-by-side comparison between Gyrolab CHO HCP assays and a reference ELISA. Two Gyrolab products were evaluated; Gyrolab CHO-HCP Kit 1 and CHO HCP 3G solution for Gyrolab, using several different examples of monoclonal antibodies (mAbs) and fragments

expressed from CHO cell lines. The CHO HCP ELISA kit from Cygnus Technologies (catalogue #F550) was selected as reference (Table 1). The Gyrolab CHO HCP 3G utilizes the exact same antibody reagents as the reference ELISA and is therefore an ideal candidate for comparison between the methods.

Table 1: Reagents, consumables and labware for CHO HCP assays

	Gyrolab CHO-HCP Kit 1	Gyrolab CHO HCP 3G	Reference
Technology	Gyrolab	Gyrolab	ELISA
Gyrolab® disc	Gyrolab Bioaffy™ 1000 HC	Gyrolab Bioaffy™ 1000 HC	-
Kit	Gyrolab CHO-HCP Kit 1	CHO HCP 3G Solution for Gyrolab	CHO HCP ELISA 3G (F550-1)
Antibody reagents	Polyclonal biotinylated goat anti-CHO HCP Capture, polyclonal anti-CHO-HCP Detection from Cygnus Technologies		

The authors used a well-studied mAb (case 1) to qualify the Gyrolab method and ascertain that all acceptance criteria were fulfilled. The Gyrolab CHO HCP Kit 1 and 3G assays delivered robust and consistent results with a limit of detection (LOD) of 3 ng/mL which matched the ELISA reference assay (LOD 3 ng/mL) and met the sensitivity requirements for low-HCP samples. Next, they performed a side-by-side comparison between the Gyrolab CHO HCP methods, either kit 1 or 3G, and the CHO HCP ELISA for several biologics (case 2-7) from the Sanofi project portfolio, including mAbs, fragments and multi-specifics. The results obtained with the Gyrolab assays showed excellent correlation with the

reference ELISA for samples extracted from different stages of the purification processes (Figure 1). For one of the example proteins (case 5) linearity issues were observed for both the Gyrolab and ELISA assays and the Gyrolab assay was found to overestimate the HCP content. The authors suggest that this was caused by very high concentrations of a co-purifying HCP. After changes to the purification protocol the linearity and agreement between assays improved. The authors concluded that the Gyrolab assays provided an efficient high-throughput analytical support for the process development studies for all targets tested (Figure 1).

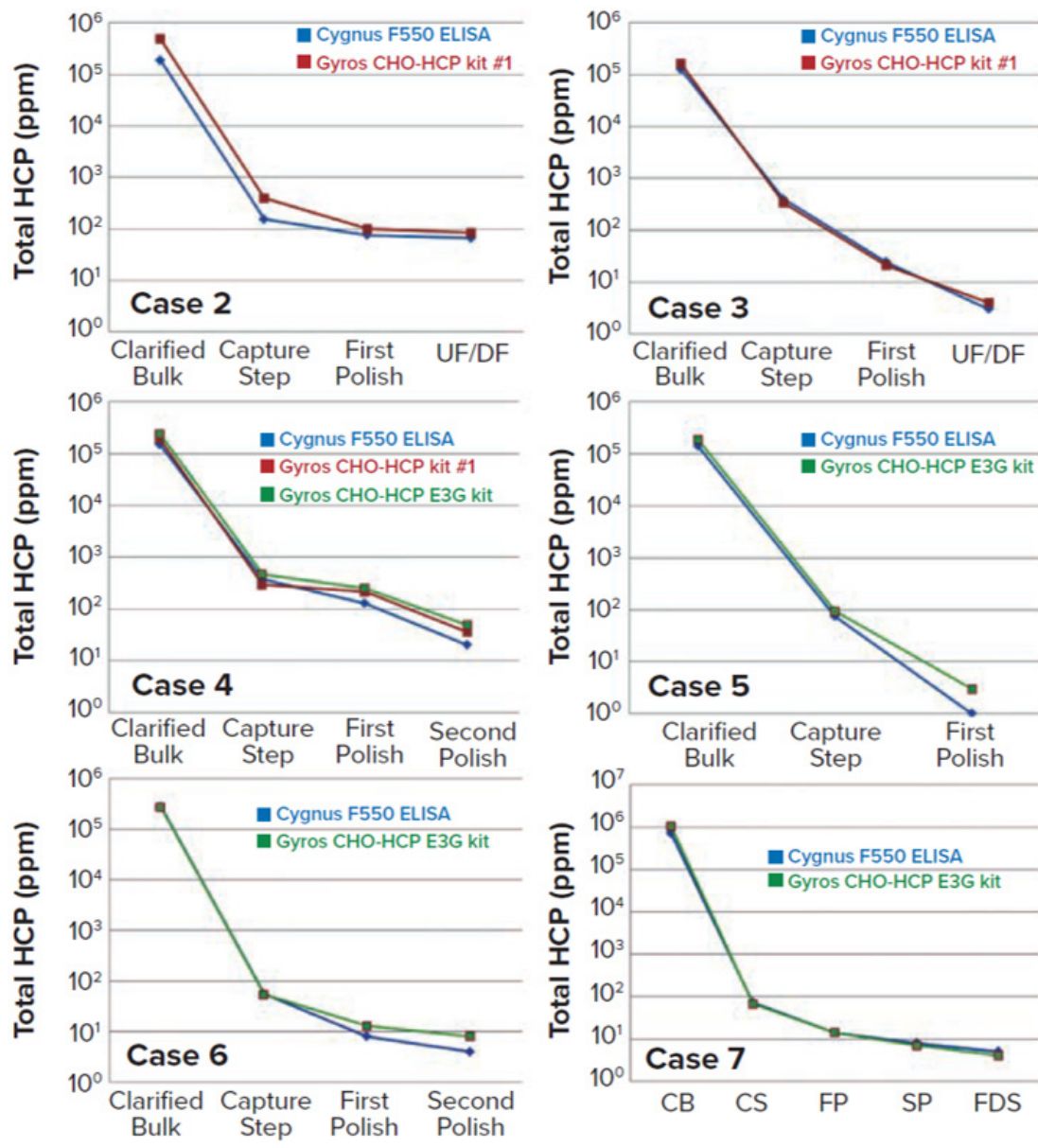


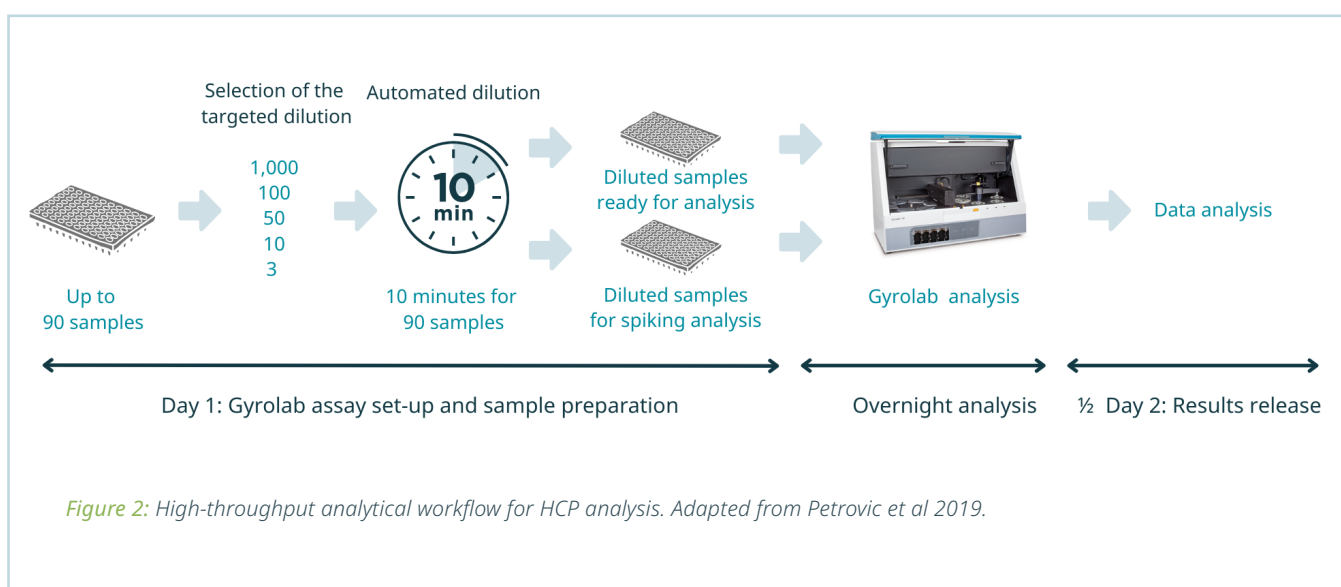
Figure 1: Comparing the Gyros CHO-HCP kits with Cygnus F550 ELISA kit results for cases 2-7. Petrovic et al 2019, printed with permission.

Abbreviations: CB, clarified bulk, CS, capture step, FP, first polish, SP, second polish, FDS, final drug substance, UF, ultrafiltration, DF, diafiltration

Automation of liquid handling to increase throughput

The Gyrolab assays demonstrated comparable results to the reference ELISA method with excellent dynamic range, repeatability and precision, and significantly increased the throughput, but the manual preparation of sample plates remained a challenge. The 5-disc Gyrolab system can run a sequence of 480 analyses overnight. However, the runs require significant sample preparations and to increase the throughput and avoid errors associated with manual sample preparation they applied an automated Bravo liquid handling system (Agilent Technologies). The comparison between the manual and automated sample preparation and

subsequent Gyrolab runs carried out by three different operators on three different sites showed very little variation between manual and automated sample preparation, with an intermediate precision coefficient of variance (CV) of 7-8% for all preparations. The resulting automation process allowed them to prepare 90 samples in 10 minutes for a given dilution and also multiple dilutions could be achieved quickly. This resulted in an automated HCP workflow that allows the teams to analyze and release results within a day and a half with instrument setup and dilutions on day 1, run the Gyrolab system over night and handle results the next morning (Figure 2).



Evaluation of cross-site CHO HCP testing using the Gyrolab platform

The publication states that Sanofi has selected the Gyrolab platform to support HCP detection on all their biologics development sites worldwide, as the use of a single platform facilitates project transfer across sites and ensures continuous support. Before implementation the reproducibility of the Gyrolab assays was evaluated. The same samples were assayed at the three development sites and analyzed using a harmonized procedure. The results showed that highly comparable data was generated from the three development sites for all tested projects and all process development steps (Figure 3).

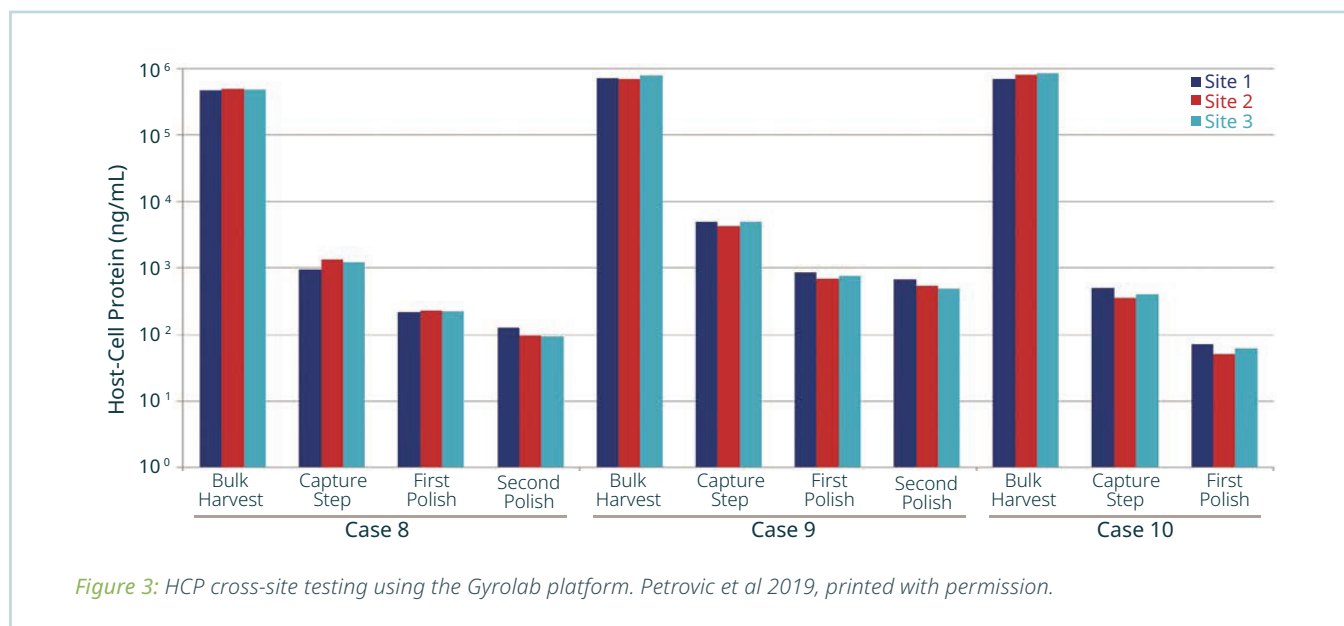


Figure 3: HCP cross-site testing using the Gyrolab platform. Petrovic et al 2019, printed with permission.

Summary and conclusion

The combination of high-quality polyclonal anti-CHO antibodies from Cygnus and Gyrolab microfluidic, nanoliter-scale automated immunoassay platform from Gyros Protein Technologies provides an HCP detection assay with a broad assay range, using low sample and reagent volumes and a fast turnaround time. High-throughput HCP analysis using this immunoassay format is fit to support the development and production of antibodies and other therapeutic proteins to meet requirements from regulatory

bodies, such as the FDA. The robust method allows for detection of even small changes between steps in the purification process. This study also shows that HCP assays can be run in high throughput across development sites with highly reproducible results, independent of operator and site. This highlights the robustness of the Gyrolab platform and the seamless transfer of assays across sites enables secure data delivery without interruptions.

References

Petrovic S, Fromentin Y, Pelluet P, Nombret J, Jegat M, Dubois H, Borges L, Kreiss P, Laborderie B, Hincapie M, Kirsch M, Karetzky L, Unger S, Schoch A & Duhau L (2019) HCP analysis to support downstream process development. *BioProcess International* 17(11-12)

United States/Canada Gyros US Inc

Toll free: +1 877 433 9400

Tel: +1 908 755 0011

Fax: +1 908 755 0001

Europe

Gyros Protein Technologies AB

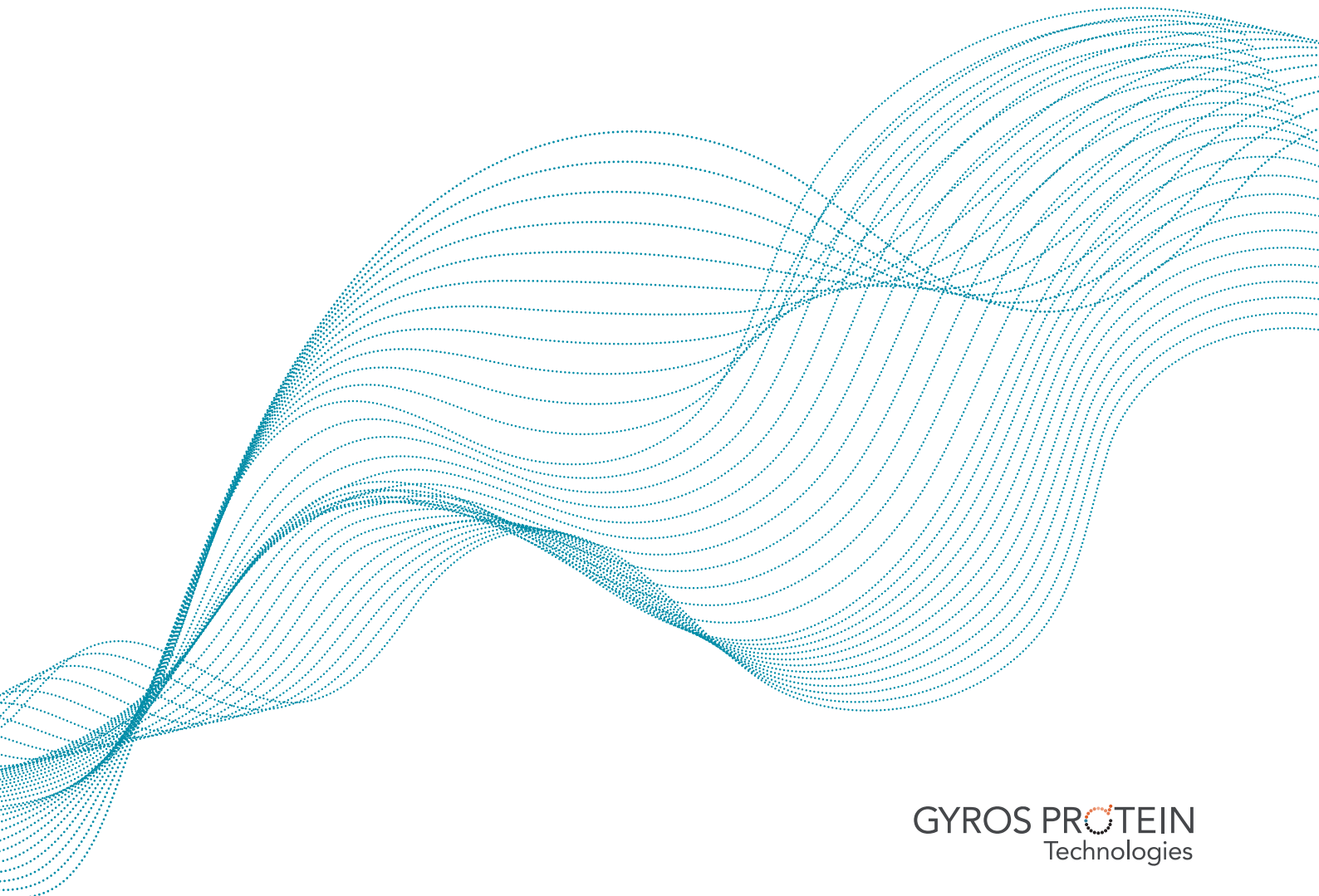
Tel: +46 (0)18 56 63 00

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information@gyrosproteintech.com

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