When is a process specific HCP ELISA assay required for HCP monitoring?
Case study on the impact of cross-reactivity against the drug

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Introduction

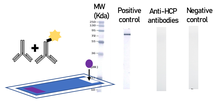
A critical aspect of the bioproduction processes is the removal of host cell proteins (HCPs) in order to minimize risk for patient safety and drug efficacy. Regulatory authorities require these HCPs to be monitored and quantified using the ELISA sandwich gold-standard method. To quantify HCPs, a generic kit is usually used during each phase of the bioprocess. But from Phase III, it is strongly recommended to develop a process specific HCP ELISA kit. Another scenario may require to go with a process specific kit : when the generic kit is no longer well adapted, because of a deficient coverage or, as discussed here, because of the cross reactivity against the drug substance. Indeed, for a generic kit, it is mandatory to demonstrate that the kit is suitable for specific process by testing ELISA parameters and determining the coverage percentage/rate of the anti-HCP antibodies in the HCP mix. The coverage is an estimation of the HCP percentage/proportion that can be detected, or covered, by the anti-HCP antibodies (Ab). This analysis is required by regulatory authorities (FDA, EMA , ...). But the ELISA parameters are also a very important control to demonstrate the suitability of the generic ELISA kits. Regarding those parameters, usually several analysis are performed like dilution linearity, spike recovery or sensibility. Nevertheless, the cross-reactivity of the anti-HCP antibodies, contained in the generic ELISA kit, must be tested against the drug to ensure the robustness of the HCP monitoring. Cross-reactivity has been proven to be a huge point in several projects, particularly with CHO cells. Custom-protocols, in ELISA and WB 1D format, have been implemented for the analysis of this parameter. The final aim is to decide if the development of a process specific HCP ELISA assay is necessary.

Cross reactivity protocols

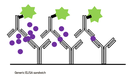
For testing the Cross Reactivity (CR) several tests are made : Western Blot 1D and ELISA. ELISA parameters involve the minimal required dilution (MRD) determination and linearity calculation. It is important to perform several techniques in order to validate the cross reactivity. Taking altogether, the data will argue or not in favor of cross reactivity with the HCP produced antibodies against the Drug Substance (DS). To be performed the DS, HCPs from mock cell Line (or purification flowthrough), the DS buffer and the anti-HCP antibodies are required.

Preliminary investigations: To highlight the CR of the anti-HCP antibodies against the DS, the first tests to be done are a Western Blot and an ELISA with a commercial generic HCP ELISA kit.

Western Blot: the DS is loaded on gel.

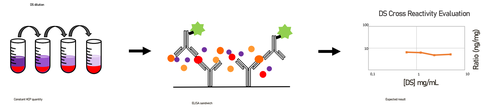


ELISA with DS: the DS is added in dilution on a generic sandwich ELISA kit



Global investigations: when previous tests do not give clear results about a potential CR, more precise and extensive investigations are launched : MDR determination and linearity calculation

Minimal Required Dilution: the MRD for the DS should be established by spiking the HCP standard in sample dilution series. The protocol is to add a fixed amount of HCPs and to dilute the DS.
With MRD determination in DS, we obtain another indication about the CR: if no dilution enables to recover HCPs, an interference of the DS can be considered.



Linearity: The ability to measure analyte concentrations along HCPs range will be confirmed using DS MRD. To confirm lack of CR of the anti-HCP antibodies with the DS, HCP accuracy is evaluated in the DS and accurate spike recovery demonstrated over the range of the assay

Expected result

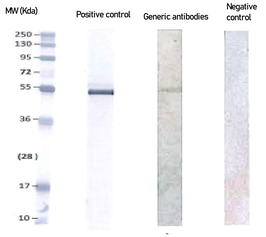
Naked Anti-HCP antibodies | Labeled Anti-HCP antibodies | Detection antibodies / Anti anti-HCP antibodies | DS | HCPs

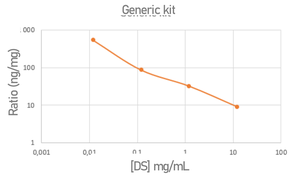
Results

Following this pattern of tests, several HCPs have been evaluated. They present different results of cross reactivity levels, which are more or less obvious to distinguish. This variety of levels highlights the interest in developing specific ELISA kits

Case study n°1:
- CHO host cells
- Cross reactivity suspected due to customer results during the monitoring
- Generic antibodies

Preliminary investigations:
Western blot is performed with the DS and a negative control using generic antibodies
ELISA is performed by testing DS in serial dilution a generic ELISA kit





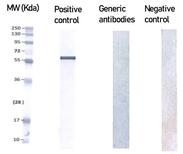
Western Blot → A band at DS molecular weight is observed

ELISA → the OD in ELISA decrease when adding DS

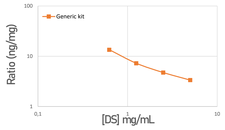
Conclusion: The CR seems to be obvious, no need to pursue with further investigations. The customer decided to launch a process-specific to ensure program good monitoring

Case study 2:
- CHO host cells
- No data about cross reactivity
- Generic antibodies

Preliminary investigations:
Western blot is performed with the DS and a negative control by using generic antibodies
ELISA tests were performed with serial dilutions of DS.



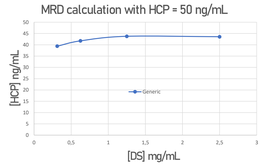
DS Cross Reactivity Evaluation

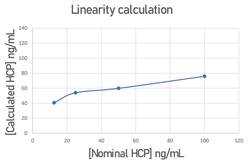


WB → band at drug molecular weight suspected but unclear situation
ELISA → a slight decrease is observed but not obviously

Decision of complementary analysis by MRD calculation

Global investigations:

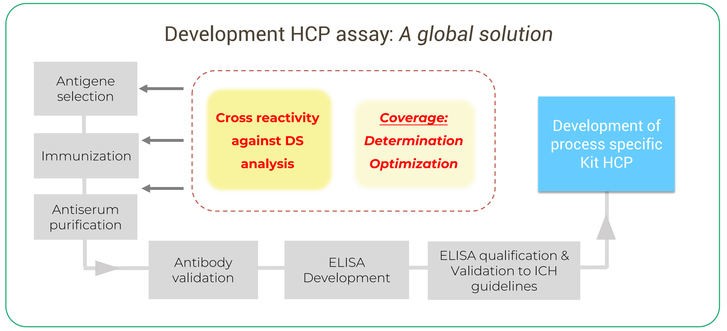




MRD → HCP standard solution is spiked in a DS serial dilution and assayed in ELISA sandwich
Linearity → Different amount of HCP spike in DS

Conclusion: The CR is not obvious. To secure and de-risk the project the customer has chosen to develop a process specific ELISA

Steps for a Process specific ELISA Kit development



The development of Process specific is time consuming, and challenging. To avoid the cross reactivity, the only solution is in implementing some controls throughout the immunizations process.
What to do when cross reactivity is detected During the immunizations?

- Block human IgG epitopes with anti-human IgG (ELISA) → Would argue for cross-reactivity of IgG with anti-HCP
- Purification process set-up with DS → Would argue for an effective co-purification DS + HCP
- DS immuno-capture with an anti-DS, staining anti-HCP (ELISA) → Would argue for a strong interaction between DS and HCP

The regulatory's requirement for ELISA kits development : **FDA and EMEA for bioanalytical method & USP1132 and ICHQ2R1**

Case study n°1 (pursue):
- Immunization of 20 rabbits
- Indirect ELISA & WB1D of the rabbits pre-immune response
- ELISA & WB1D for each bleeding during immunization

Preliminary results & Strategy:
CR against the DS is detected

Process of affinity chromatography to deleted the CR

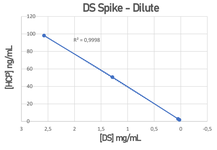
Test of cross reactivity-deleted rabbits pAb generated in specific ELISA kit

The results obtained show no anomaly, results are same as theoretical expected:

no cross reactivity detected in the specific kits

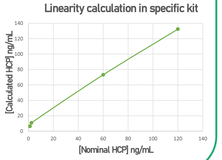
Test n°1: ELISA

HCP dilution in each well
DS = 2,57 mg/mL + HCP = 100 ng/mL
Dilutions with dilution buffer



Test n°2: Linearity

DS = 1,2 mg/mL
Spike HCP 4 concentrations
120; 60; 2,4; 1,2 ng/mL



Conclusion

The cross reactivity against the drug of anti-HCP antibodies contained in the HCP ELISA KIT has a huge impact in the monitoring of the HCPs. Any cross-reactivity of the anti-HCP antibodies with the DS may compromise the test method and yield biased results. According to the FDA Guideline Residual Host Cell Protein Measurement in Biopharmaceuticals), the contamination of HCP antigens with DS must be avoided to prevent generation of anti-product antibodies. Hence for each anti-HCP antibodies (generic or specific) an adapted protocol has to be set up to measure it. For every anti-HCP ELISA kits antibodies (generic ones as specific) an adapted protocol has to be set up to measure them : Western blot with the drug coated and ELISA testing. These tests can be performed in parallel. In case some cross-reactivity is detected and confirmed, then there is no other choice to switch to a process specific ELISA kit. The same vigilance for potential cross reactivity induced during the immunization program is required. The cross reactivity can be detected by both protocols presented in this poster (WB/ELISA). Then, different serum purification strategies can be set up and performed in order to eliminate the cross-reactivity