Table 9: Host Cell Proteins – Reagent Coverage, Identification and Risk Assessment

Session 1:

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Session 2:

Facilitator: Lisette Coye, *Seattle Genetics, Inc.* and Dan Kenney, *Rockland Immunochemicals, Inc.*
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SCOPE:

Host Cell Proteins (HCP) are process related impurities found in many biological products. Clearance and monitoring of HCPs are a critical aspect of drug substance manufacturing. Health Authorities are focused on analytical methods to detect HCP including coverage. In addition, risk assessments of known HCPs may be required to support root cause analysis for a variety of issues. Please join the HCP roundtable to have a general discussion on these and other HCP related topics.

QUESTIONS FOR DISCUSSION:

1. Host cell protein detection can improve as new ELISA bio-reagents are needed. This can lead to improved coverage.
    a. How do attendees evaluate ELISA coverage?
    b. Is there any phase appropriate strategy (commercial kit vs in-house assay, technologies/methods for reagent coverage, etc.)?
    c. If more HCP is detected, it is possible that product specifications need to be widened. How have attendees handled regulatory implications of widening specifications?
2. ELISAs are the most common analytical method for detecting HCP. However, industry is starting to apply orthogonal approaches including Mass Spec (MS). Have attendees received request from regulators to file MS results?
3. Identifying HCPs would be needed as part of a root cause analysis (safety signals, HCP ELISA related issues or process and product quality concerns).
    a. Have attendees received requests from Health Authorities to identify HCPs in products?
    b. How are companies handling risk assessments for identified HCPs?
4. How are attendees screening for lipase activity? Are there strategies that can mitigate risk early in development?
5. How are attendees setting specifications on the Drug substance for newer modalities (which may be more complex than MAbs)
    a. How is the risk analysis being handled?

DISCUSSION NOTES:

Session 1:

1. Host cell protein detection can improve as new ELISA bio-reagents are needed. This can lead to improved coverage.
    a. How do attendees evaluate ELISA coverage? *How much reagent coverage is needed? Two approaches are used: 1. Apply sample to affinity column, label load and eluate proteins with different color, run on a gel to compare coverage. 2. More traditional Western Blot*
    Generally, >50% coverage is needed.

One case: company used a commercial kit to achieve ~50% coverage. Regulatory agency requested improvement to switch to process specific assay. In-house assay (improved plate washing) was developed to achieve 65% coverage and accepted by the agency.

b. Is there any phase appropriate strategy (commercial kit vs in-house assay, technologies/methods for reagent coverage, etc.)?
Early phase—commercial platform kit; Phase 2—process specific assay.
Good coverage kit can be used from early to commercial stage.

Kit made with HCPs from cell Lysis vs supernatant----choose the kit that is more representative of the process.

c. If more HCP is detected, it is possible that product specifications need to be widened. How have attendees handled regulatory implications of widening specifications?
One case: Spec was set at 20 ppm. LOQ of the ELISA kit was 0.2 ppm. Spec was lowered to 10 ppm due to process capability.
Second case: Increased spec (<100 ppm) due to kit change during IND. Agency accepted.

2. ELISAs are the most common analytical method for detecting HCP. However, industry is starting to apply orthogonal approaches including Mass Spec (MS). Have attendees received request from regulators to file MS results?
50% attendees use mass spec for characterization, not routinely, only for investigation.

No request from regulators to file MS results.

Identifying HCPs would be needed as part of a root cause analysis (safety signals, HCP ELISA related issues or process and product quality concerns).

a. Have attendees received requests from Health Authorities to identify HCPs in products?
Case specific for investigation only.
b. How are companies handling risk assessments for identified HCPs?
Identified for process improvement. No risk assessment needed if cleared at the DS step.
Common risk assessment approaches: Literature research, in-silico assessment/prediction and in vitro studies.

3. How are attendees screening for lipase activity? Are there strategies that can mitigate risk early in development?
One case: lipase amount was measured during Phase I development.
No lipase activity test is implemented.

4. How are attendees setting specifications on the Drug substance for newer modalities (which may be more complex than MAbs)
a. How is the risk analysis being handled?
One case: Spec was set higher than 100 ppm using commercial kits with dosing justification. Spec was accepted by agency at phase I.

Is anyone using more than one species to get better coverage? Preferred animal?
Rabbits, goats are being used but no feedback that anyone is mixing species

<u>Session 2:</u>

1.

a.

- GSK do 2D western blot to evaluate coverage. Others don't; use immunoaffinity chromatography as is mentioned in ICH and is more sensitive; this has a lot of advantages as the HCPs are in solution and Ab and antigens react natively, rather than immobilized on a gel.
- But affinity chrom methods normalizes the pH and conditions so this may alter affinities of some in the mixture
- May be better to use a blend of both to supplement your data set. Negative controls are so critical in both methods.
- Generation of own antibodies is a tricky area where there are no custom kits and services available in the market. The cell types and lines being used are evolving year on year.
- Smaller companies still relying on kit-based methods; choosing the proper commercial kit is difficult. Lot that goes into selecting the correct assay; there is no correct answer but which is most appropriate for you. Supplementing the kits with MS studies at early stages can help this. Your manufacturing technology can help inform your selection; Lysate kit or supernatant kit etc? what's your viability of cell lines at your time of harvest - can select kit based on this type of product, or do I need to develop a process-specific. One size does not fit all!
- The Ab generated should be very reflective of your process; need to match the Ab to antigen i.e. Ab manufactured in supernatants for similarly generated antigens. But the variety of manufacturing techniques now available adds complexity. Null cell culture needs to be obviously generated in same way as your drug manufacturing - coverage must obviously be process specific.
- 'Jackpot proteins' - can hijack and overreact in your assays, those HCPs may need to be stripped out and use specific assays for that jackpot protein. If you understand more about that protein can utilize its pI/size etc to handle it.

b.

- Maybe you'll get -Expectation used to be that at phase 3 you need to be utilizing process-specific assay; but now if we use newer tools we can correctly assess the best assay. Utilize your extended characterization data to support your decision; but remember to make the risk-based decision about where your 'control' lies.
- Do you want to own and control your reagents moving forward? Up to you to decide what spend level you want!
- Some of the kit vendors struggle to service customers with these needs - consistency over long periods of time is critical on the kit-based front.
- Manufacturers implementing process changes can throw a spanner in the works here; tightened timelines are not possible for the kit manufacturers when they are trying to service multiple customers.
- Need buy in from the agency on your approach if you are going to stay kit-based methods
- Switching reagents in the kit-based space; most companies see no change, some see higher/lower responses which may knock out specifications.
- One company generated in-house custom Ab, had same one previously from Cygnus. Made a new one in-house and had to do a bridging study due to differences in results between the two. The coverage was higher so more sensitive Ab detecting more HCPs overall; used other historical projects on the old Ab to show similar increases across other programs also. Used retains from old batch to test with new Ab to again show that there was no fundamental change in process.
- Need to make sure that the retain samples have bene stored correctly!! Example given of someone storing samples at 2-8C for 5 years
- Need to think about how you will approach the agency with this change also; come with a complete dataset with demonstrably evidence. Using the
- We have the ability to:

    - MS - we know a lot of the problematic proteins, Cygnus use AAE to enrich the problematic proteins and compare old v. new reagents; gives weight to the data you need to switch reagents.
    - The concept of ng/mL results not well understood by those outside the field, it's an Immunological equivalence test!

2.

- EMA are almost requiring it for blood products. Have requested western blots, but not MS yet.
- It's being used a lot to identify known contaminants, shows you are using good techniques to characterise your molecule.
- If you have a specific assay for your known problematic HCP that may be enough for regulator.
- Most companies just figuring out what to do, expectation is that you perform risk assessment - is it biologically active (you may have lots HCPs, but is it a safety problem)
- Have you sufficiently characterised your process to know that It's safe.
- MS may end up the expectation, becoming more common and being talked about at a lot of conferences and meetings.
- Hitchhiker HCP identified through MS; developed method to detect, and showed that it is cleared through the process. Don't need to analyse routinely once you can show it's cleared. The dilutional linearity study identified this, and the formulation group/stability were seeing PS80 degradation - this prompted them to investigate.

3.

- Most companies coming with this info themselves. The problem will be showing up earlier than the stage when you get to regulator.
- Risk assessments:
    - One example: it’s a helpful approach but FDA can still be conservative and require a specific assay to control particular HCPs even though the tox and clinical data supported the removal of the assay. (this HCP wasn't seen at small-scale, only when they got to large-scale).
    - Feeling is that the agency is still very concerned and sensitive to HCP-related issues and data.
    - The advent of data linking poor clinical outcomes to HCPs in the early 2000s has kept this topic alive in regulators mind.

4.

- One example: if not having dilutional linearity issues they don't go down the rabbit-hole. Feel there is no need to identify every single one. But the challenge is that if the dose increases will see a problem.
- Dilutional linearity is such a critical and informative test - most powerful tool we have to determine if there is an issue
- Other option is for the company to at least know all the info about their HCPs - you don't have to share the info with the agency. Often the company reaction can be that we don't want to know the data! Probably should try to learn this as early as you can in the process.
- Having the lipase assays can be really useful for the downstream process engineers; can help them design a better process at development stage. Using the generic assays for this can be great.

5.

In gene therapy, have no idea really yet what to do in this field - host cell DNA often is more of the issue here than HCPs.

How is the risk analysis of this being handled?

How do you generate a null-specific Ab?

- Standard approach of generating null cell line without the drug code in it.
- Need to ensure that the creation of a process-specific assay is handled in the right section of your company, need an in-house expert.
- Can engage vendors to create process-specific Abs; but all about engagement with the vendor.
- CHO can generate so many different proteomes due to different ways it is being used.

What is good coverage?!

- Some like to see 60% or higher but depends on the cell line. Can justify if between 50-60%. (this is based on spot-counting which is obviously very subjective)
- The methods that you use to determine coverage are often more variable so can get 50% or 80% results! Informally agencies have said that it needs to be broadly reactive to at least the majority.
- Any experience with early stage proof of concept in animal models?
- A lot of companies are using platforms e.g. CHO specific etc. But how good is the platform? There are quite a lot of things which downstream can do to help with the clearance of problematic proteins.