Table 20: Trace Metals

Facilitator: Maura Kibbey, *US Pharmacopeia*

Scribe: Hua Wang, *US Pharmacopeia*

SCOPE:
Design and optimization of cell culture media is critical to the yield, quality, and consistency of biotech products. Numerous studies have demonstrated that certain trace metals are tied to certain critical quality attributes (e.g., copper and manganese in relation to glycosylation) so control of levels during upstream operations can greatly affect the production process. Chemically defined media can be purchased premixed or components individually characterized and mixed to specified levels. Given that suitable ranges for different trace metals can vary tremendously, participants in this roundtable will share and brainstorm on best practices for raw materials containing trace metals for cell culture production and analytical techniques that can be most easily applied for reliable answers.

QUESTIONS FOR DISCUSSION:

1. Do you confirm the quantity of each trace metal of interest in your cell culture media? As single components?
2. How large is your sample size relative to the size of your raw material lot? Do you test multiple samples from the same bulk lot?
3. How many trace metal levels do you control? Which ones are most critical?
4. Are you able to use one detection method across all metals of interest? If so, what is your method of choice?
5. Do you need to do sample extraction prior to detection? If so, what types of controls do you use and what is your biggest source of matrix interference?
6. Have you implemented additional mitigation steps with the media manufacturers to control contamination coming through the supply chain of high risk raw materials?

DISCUSSION NOTES:

Participants of this round table discussed best control strategies, other people's experience, and concerns dealing with trace metal issues.

For the control of trace metals, most participants said that they confirm trace metal levels in both cell culture media and component raw materials. Participants mentioned they usually use in-house generic ICP-MS method protocols to monitor trace metals. The most common elements that were mentioned and monitored were copper, manganese, magnesium, and some transition metal ions. The participants noted they normally don't use any special sample extraction/preparation prior to detection.
No one was able to share a successful story of how they control the purity/consistency of raw material or standards by communicating with vendors.

Since the sensitivity of cell lines/processes to trace metal varies, performance testing with their cell lines can be just as important as other standard tests (e.g., purity) so adding many additional incoming tests can add risk of rejecting batches that may not affect production. Some participants

mentioned they usually don't trigger trace metal related investigations unless they see an impact on the quality of downstream batches (failure or affected product quality). Some mentioned that in early stages of development, the fast pace/turnaround cycle usually doesn't support time for a lot of extra work; however, if production is affected then additional tests will be added. Some agreed that it may be necessary to purposely test these hypotheses to understand their impact to products. Some participants felt that some reports of impact of certain trace metals on quality attributes were working with extremely low amounts of trace metals so they were more sensitive to change. Many agreed that when working at more "typical" levels that they usually don't see a lot of variation.

When raw material/excipient standards exist, all agreed that they follow the typical compendial standard. For content uniformity, participants agreed that the testing strategy for large sample lot sizes can be more challenging than small lot sizes. Some noted they use statistics-based rationale to determine how many replicates or numbers of containers to be tested.

The group also brought up some related excipient concerns. One participant comments that the oxidation/degradation of polysorbate induced by metal ions may be more important than some of the compendial quality tests of the polysorbate itself (even at ppb level). Participants discussed possible approaches to mitigate the issue, such as adding chelators. Some mentioned that trace metals in media can induce protein oxidation, impact glycosylation, affect the level of reduced thiol groups in monoclonal antibody products.