Table 5: Replacing in vivo with in vitro Potency Assays

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SCOPE:
In vivo methods have played a central role in controlling the potency of vaccines. However, the in vivo methods present inherent limitations, such as high variability and complexity linked to the use of animals. Additionally, there is a global commitment to the reduction of animal usage wherever possible in accordance to the 3Rs principle (Refinement, Reduction, Replacement). As a consequence, in the recent years, efforts have been directed towards the development and implementation of in vitro methods to replace licensed in vivo assays. In general, in vitro methods combine a lower variability and higher sensitivity, allowing a better evaluation of vaccine potency at release and during stability testing. Due to the inherent assay variability of the in vivo methods, demonstration of a correlation between the in vivo and in vitro methods may be extremely challenging, requiring extensive efforts. Nonetheless, the in vitro method could be more appropriate in detecting changes in the product profile relevant to monitoring potency attributes, despite the lack of correlation with the in vivo method. Participants in this roundtable discussion are encouraged to share their experiences and approaches for replacing in vivo with in vitro potency methods and any feedback received from Health Authorities.

QUESTIONS FOR DISCUSSION:
1. Which are the alternative approaches to be followed when it is not possible to show one-to-one comparison between the in vitro and in vivo methods due to low discriminability and/or high variability of the in vivo assay?
2. Is it adequate and sufficient to show the ability of an in vitro method to distinguish between potent and sub-potent vaccine?
3. Is the demonstration of the functional relevance of the target of the in vitro assay sufficient to enable replacement?
4. How can the different sensitivity between in vitro and in vivo methods be used to support the concordance between the two methods?
5. Does the presence of adjuvants, e.g., an aluminum salt, complicate the ability to replace an existing in vivo assay with an in vitro assay? If so, how may this complication can be addressed?

DISCUSSION NOTES:
There were ~25 people at the table (in two rings) and it was very hard to hear everyone due to the number of people and the general level of noise in the room. In addition to the main table there was also an overflow table that discussed the same topic. Main points from the round table were:

1. Consensus of the group was that it may not be possible to show a correlation between in vitro and in vivo assays
2. Based on #1, it was agreed that a correlation was not then an absolute requirement to replace an in vivo assay with an in vitro one.
3. The burden falls on the in vitro assay to show a link to the mechanism of action of the therapeutic that is being tested.
4. Adjuvants in vaccines make it more difficult to establish the link between the two assay types. In this situation, companies may need several assays working together (i.e. need to characterize the adjuvant and the antigen).
5. In the case of several companies trying to develop similar assays for the same product, it was suggested that setting up a consortium would help enable standardization. There may also be a role for international standards in those cases.

6. Force degraded material can be very helpful in the demonstration of the importance of the in vitro assay in some cases.

7. Some older in vivo assays were never correlated to responses in humans and this could provide another approach to replacement if there is new information about the mechanism of action.

8. In general it was accepted that in vitro assays are more sensitive than in vivo assays and this provides advantages for certain applications of potency assays.

9. Demonstration of the higher sensitivity of the in vitro assays in detecting sub-potent lots or changes in critical attributes of the product might be needed to support the lack of a direct correlation between in vitro and in vivo potency assays.

10. In the cases where correlation between assays is required/attempted, the reagents and sample sets used in those studies are of critical importance.

11. It was suggested that a main driver from some companies was the perception that many good lots are discarded due to spurious results in animal potency assays.

12. It was acknowledged that replacement is mostly a unique situation and should be handled on a case by case basis. There may not be a lot of commonality across the situations.