Table 47: Potency Tests for Vaccines: Hard to Develop or Change?

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SCOPE:

A test for “potency” is one of the required, listed tests (21CFR610.10) and which “shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in 600.3(s). 21CFR600.3(s) defines “potency” as “the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.” In other words a potency test shows that the sample/lot/batch is “active”.

Most potency tests for vaccines were/are quantal in vivo animal tests. Some predate even the FDA or CFR. Most of these vaccine potency tests were detailed in older versions of the 21CFR. More recently researchers in academia, industry and FDA have developed vaccine tests that quantitate the level of neutralizing epitopes in a sample (e.g. by immuno-, mass spectrophotometric or cell based tests). However, few of these newer formats have been approved by the FDA and are in use.

QUESTIONS FOR DISCUSSION:

1. What vaccines are made or are in the process of being developed by your company or group?

2. What type of tests are being run/developed for each vaccine?

3. For legacy products: Has your company considered replacing the test format with a new (faster, easier, more reliable) format? Which format(s)? What problems have you encounter?

4. If your company is still using the existing test format, what are the major problems? What has stopped your group from trying a better format?

5. For new or recently approved vaccines:
   a. What potency test format did you choose? Why?
   b. What do you like about the format? What are the problems with it?

6. Has the approval process for the new/changed format gone as expected? What do you wish you knew before you started the process?

NOTES:

The participants at this round table varied from novice to those highly experienced with vaccine potency assays and ranged from small to large companies, consultants and CBER. Product experience ranged from legacy viral and bacterial vaccines to state-of-the-art vaccine products. To frame up discussions on potency
assay changes, the group agreed to keep the 21CFR 600.3.5 definition of potency as the key guiding principle. Specifically, “The word potency is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result...” First, potency assays need to meet these criteria. Secondly, it is critical to identify why a potency assay change is needed, understand the risks and benefits, and prioritize conflicting change drivers. In cases where the mechanism of action is not well understood, it has sometimes been better to leave a potency assay “as is”, despite a desire to modernize the assay or comply with the Replacement, Reduction and Refinement (3Rs) initiative to move away from methods that use animals. For legacy products, replacement assays have been investigated but there don’t seem to be apparent gains. For legacy products, sometimes potency is the only tool available to monitor a product quality change. With these as foundational principles, some case studies experienced by the participants were discussed.

**Case Study 1**

A legacy vaccine potency assay, consisting of inoculating agar in a tube with sample and manually counting resulting colonies, was developed in the 1950s, is still performed the same way today and has its own CFR chapter. The assay had not been well characterized and what is known is mainly based on tribal knowledge and not concisely documented.

Following scale up and site changes, increased invalid rates for the method were observed. The method was judged to have high variability at a 43% CV. It was noted by the group that a high %CV is not necessarily unexpected or a “deal breaker” and a 40% CV is not bad for legacy products, especially since many are plotted on a log scale (e.g. for plaque assay results). However, the sponsor’s concern was that assay variability compromised the use of the potency assay as a tool for assessing potential effects of process changes. The goal was to reduce method variability to approximately 20% CV, revalidate the assay and file for approval in time to support analysis following a tech transfer.

The group questioned whether method remediation for lot release was really needed in this case if the assay is meeting the requirement of detecting a quality attribute. Given the problem with the assay concerned its utility for assessing process or site change effects, it was suggested to develop a more precise assay for product development and comparability purposes and not necessarily for lot release and the specification. It was discussed whether vaccine developers should move to a two-assay approach with one assay to detect and characterize potency changes following process changes and antigen mapping for lot -to-lot release. It was acknowledged by participants that this may be the direction the industry in heading and CBER is open to this approach.

The question was raised that if the precision of the potency assay is improved, how is data generated by the new assay compared to historical data from the legacy potency assay with the wide %CV. It was suggested that assays could be designed to look at this. For example, side-by-side analysis by assays can be performed, but it was acknowledged it can be difficult to get samples and to get QC labs to run these “development” assays. There are strategies for testing released lot samples that do not put released lots at risk. For example, you can lay down “development” samples but it was noted that this can be risky. It’s possible to blend two released lots for testing and this has been acceptable to FDA. Clinical data can be generated during the
dose ranging studies to allow for a correlation of drug potency to support CQA. Forced degradation samples can also be examined.

The actual approach taken by the sponsor was to gain an understanding of and document method performance and characterization and then use what they learned to improve method performance per their goal above.

**Case Study 2**

Sponsor is looking to reduce animal use but sometimes animal based assays are the most sensitive assay. There is a barrier to completely removing all animal based assays, especially for black box legacy products for which there is not a good understanding of the mechanism of action and characterization of the critical quality attributes for immunogenicity (as an example).

In some cases, animal potency assays can be replaced if the vaccine relevant epitopes are well defined and can be monitored. For complex products and/or vaccines in adjuvants (aka whole system) there is not always a good replacement for animal potency assays. This is because desorption of the vaccine from the adjuvant can alter the vaccine properties and impact potency assay results.

But, sometimes the relevance of the animal model is not proven to be representative of human biology, as in the mouse antibody model. So antibody scans of vaccine structure could be better. But remember in the presence of the adjuvant you may have no idea what the protective epitopes are (e.g. pertussis vaccine). Also, some vaccines are so complex and/or uncharacterized that it’s not possible to base a potency test on product characterization and you have to do an in vivo test.

For modern biotech vaccines it’s been possible to move towards analysis of the 3D structure, even for adjuvanted vaccines, but it may still not be possible to definitively identify what the protective epitopes are. Some of these types of assays may measure antigenicity, not immunogenicity. Additionally, the duration of the antigen to adjuvant (e.g. alum) interaction over time is a factor that needs to be taken into account with respect to potency. For Aluminum Hydrochloride, it’s possible to do a competitive inhibition ELISA. This approach needs to be justified as representative of how the vaccine works in people and the necessary reagents have to be attainable. It was cautioned that assays using a binding matrix (e.g. plate or bead bound reactions vs. in solution reactions) can alter the affinity/avidity of the vaccine and cause spurious results.

**General Discussion Topics**

1. Regional potency testing can be problematic to reproduce. FDA testing tends to be manufacturer specific, not general to the type of vaccine or type of method; this is not the case in other regions. In vitro assays are problematic for standardized testing by Health Authorities as they are not typically standardized across different manufacturers and can be very much an art form for legacy potency assays. Additionally, problems in assay performance can be caused by subtle differences in reagents, reagent lots, and difficulty in getting the same species of animal in other countries.

2. Key questions to answer for defining CQAs when developing new live virus vaccines (e.g. oncolytic viruses with immunogenic properties):
   - The virus has to infect cells or in some cases specifically infect some cell types but not others
   - In some cases, the virus infection has to produce a protein in the infected cells
• Has to leave the infected cell in a form that allows it to specifically infect other target cells
• Have to define whether it cell type and/or species specific
• Have to identify what quality characteristics have to be controlled for safety and efficacy
• Vaccine design has to be practical

3. For multi-epitope polysaccharide conjugate vaccines, how it is determined whether all the epitopes are potent? Assessment of each individual epitope is considered a potency test. Physiochemical analysis (e.g. polysaccharide size and structure) of each epitope has been done.

4. The Rate Nephrology assay was noted as being especially undefined, but is used in some cases as a potency assay.