Table 29: Replacing *in vivo* with *in vitro* Safety Tests for Biological Products

**Facilitator:** William (Bill) Egan, GlaxoSmithKline Vaccines  
**Scribe:** Lesbeth Rodriguez, Bayer

**SCOPE:**  
*In vivo* safety tests are used during various stages in the development of biological products, from the initial qualification of cell lines to the release to market of the final product. Uses of *in vivo* tests include the detection of potential adventitious viral agents (such as with the various antibody production tests) or the detection of pyrogens (with the use of the rabbit pyrogen test), or tests for residual toxin activity in various vaccines (such as for residual diphtheria toxin in the toxoid vaccine).

Since these *in vivo* tests were first introduced in the control of biological products, various *in vitro* methods have been developed, either as specific replacements, such as the MAT as a replacement for the RPT, or, although developed more generically, may serve to replace *in vivo* tests, for example the potential use of NGS to replace many animal-based tests for adventitious agents. This Roundtable will focus on the areas for which *in vitro* tests would be desirable/advantageous and issues that have arisen in their replacement.

**QUESTIONS FOR DISCUSSION:**  
1. Several *in vivo* safety tests have been mentioned above as potential candidates for replacement with *in vitro* tests. What additional *in vivo* tests might also be replaced?  
2. Several potential approaches to the replacement of *in vivo* safety tests have been noted above. What additional methodologies might be used as replacements for the tests mentioned above or for additional safety tests noted in response to question 1?  
3. Have particular difficulties or challenges arisen in the replacement or intended replacement of various *in vivo* tests?  
4. In your experience, has the regulatory acceptance and acceptance criteria of replacement *in vitro* tests been harmonized?

**DISCUSSION NOTES:**  
- Ideally Next Generation sequencing would reflect and eventually replace many of the safety tests now in use  
- Large data set may be obtained from Next Generation Sequencing; need to understand what to do with the data  
- Next Gen could be used to monitor production for adventitious agent contamination by looking at different timepoints in the process and monitoring for increasing sequences  
- For mycoplasma testing, a hybrid approach (culture plus PCR or Next Generation Sequencing may be the best approach if sensitivity is an issue)
- Monocyte activation test (MAT) allows for the detection of both endotoxin and non-endotoxin pyrogens whereas the LAL test only detects endotoxin. Due to the complexity of productions, the European Pharmacopeia (EP) introduce the need for a test for non-endotoxin pyrogens and MAT has now been included in EP as a method to use to test for endotoxin and non-endotoxin pyrogens and may replace the rabbit pyrogen test. This method has not been recommended in USP and to the group’s understanding was not being used in biologics yet. Methods which allow detection of non-endotoxin pyrogens become more important for inherently pyrogenic products.

- In EU, you will need to demonstrate that the product does not have any non-endotoxin pyrogen to only do LAL testing

- Why are people not replacing old methods with new methods? 1) Familiarity in the US; 2) Europe has a directive to replace in vivo tests when possible; 3) some tests are outsourced and therefore, becomes challenging to convert unless testing lab does the new method as well