Table 8: Physio-chemical Methods to Replace Bioassays for Potency and Biological Activity Testing - Examples and Experience

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Scope:
In contrast to small molecule drug substances, whose activity is routinely determined by physico-chemical assays, biologics are still required to use costly, and frequently, less reliable *in vivo* or *in vitro* bioassays to determine biological potency. While this stand point is slowly changing, e.g., the use of Erythropoietin BRP CRS as a chemical reference standard by the EDQM, most agencies still remain staunch proponents of bioassays.

Questions for Discussion:
1. Would you be interested in replacing your bioassay by a physio-chemical method? Do you find physical parameters which are predictive of biological potency?
2. What experience have you had with agency discussions? Have you been given any hope of a future replacement?
3. What time frame do you see as realistic in replacing bioassays by physio-chemical methods?

Discussion Notes:

Just a few reminders about the use of Erythropoietin BPR (Biological reference Preparation) and CRS (Chemical reference Substance):

- Erythropoietin BPR is intended for use as the reference preparation for the *in vivo* bioassays.

- Erythropoietin CRS, for physicochemical tests, is intended for use as the reference preparation for the following tests as prescribed in the European Pharmacopoeia: Capillary zone electrophoresis, Polyacrylamide gel electrophoresis / immunoblotting and Peptide mapping.

- Erythropoietin CRS is also intended for use as a reference standard for SEC system suitability.

Therefore, contrary to what could have been understood in the abstract, even if the Erythropeoietin CRS is used for physicochemical testing, these tests don't replace the *in vivo*

Regarding the replacement of bioassays, all attendees were interested in and everyone agreed to say that is a real challenge: Which surrogate test(s) can reflect the mechanism of action of a biological product?

Actually it was underlined that binding tests and/or physicochemical tests have a huge potential to replace these bioassays.

Further to this statement, how must we proceed? Everyone agreed that a huge amount of work must be performed on the data collection, in order to establish a correlation between bioassays and the surrogate test(s). But it may be difficult to correlate biological activity to physicochemical assays without the additional use of a bioassay… It's why an intermediate step could be considered by implanting some binding assay (SPR, BLI); in some cases a link between binding activity and peptide mapping can be established.

How to integrate these surrogate tests in the product's life cycle? Actually two main considerations have been underlined:

- Regarding the regulatory requirements it seems be easier to implement these new tests for the launch of new product than to change a test for a commercial products.
- But the reduction of the number of animal testing is a priority for industrials (when it's possible), which can support the development of surrogate tests.

A participant confirmed that they had already been able to replace their bioassay by a binding assay as this reflected the mode of action. But the more complex the biologic activity is, e.g., multi-step the more difficult it will be to replace the corresponding bioassays with a simplified assay.