Brief Introduction of WHO Guidelines on the replacement or removal of animal tests for the quality control of biological products

IABS/AFSA meeting

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Outline

- Background of animal testing
- WHO position and ECBS recommendations
- Development process of the guideline on animal testing
- Brief introduction of the guideline

Background
-Quality control testing of biological products

- Animal testing has been a long-established tool in the development of biological products, providing critical information on their mechanisms of action, safety and efficacy. Some tests also continue to be used post-approval to monitor product quality as part of the quality control processes
- Major advances are now being made in the development and implementation of non-animal methods for the quality control of biological products, driven and guided by scientific evidence and technological innovations
- 3Rs are guiding principles for the ethical use of animals in research and QC testing
- Implementation of GMP, QMS, QbD
- Define of CQAs and monitoring production consistency

Request and challenges

- Request from WHO member states and stakeholders to develop a specific document on application of 3Rs principles into QC and lot release of vaccines and biologicals
- NC3Rs review report on WHO written standards
  - 63 out of 81 WHO documents reviewed included animal test
  - Focus on 5 areas of testing: adventitious agents, pyrogenicity and endotoxin, neurovirulence, potency and specific toxicity

Title and scope

- Title: Guidelines on the replacement or removal of animal tests for the quality control of biological products
- Scope
  - provide guidance on a range of scientific and regulatory considerations with regard to the **replacement or removal** of animal tests for the **quality control** of biological products
- Target audience: developers, manufacturers and regulators

General considerations

- Product testing should be science driven
- Review scientific value and relevance of animal test and strategy for QC testing
- Introduce the concept of consistency testing, use of combination of tests, where appropriate, to replace a single *in vivo* test
- Highlight the contribution of better product characterization in pre-clinical and product and process development
- Explain how the validation status and inherent variability of ‘old’ *in vivo* tests can limit the ability to make comparisons to new alternatives
- Use best practice in husbandry and sourcing animals
- Towards eliminating animal tests in QC testing

Specific considerations-1
-Adventitious agents testing

- Adventitious agents are contaminating microorganisms of the cell culture or starting/raw materials that have been unintentionally introduced

- Assays includes:
  - In vivo adventitious agent testing (suckling and adult mice, embryonated eggs, guinea-pigs and rabbits)
  - Test for haemadsorbing and haemagglutinating virus (RBC)
  - Test for mycobacteria (Guinea pigs, cell culture)
  - Test for avian virus

- Conclusion
  - Molecular methods (PCR or HTS)
  - Cell-line based culture test, if needed (only when positive signals are obtained when using molecular methods)

Specific considerations-2
-pyrogenicity and endotoxin testing

- The RPT was developed more than 100 years ago
- Pyrogenicity Testing
  - Monocyte Activation Test (MAT), Using human blood cell, PBMC or monocytic cell lines
- Endotoxin testing
  - Limulus amebocyte lysate (LAL) or Tachypleus amoebocyte lysate (TAL) assay
  - Alternative: rFC assay, uses a recombinant version of Factor C
  - Alternative: rCR assay: Recombinant Cascade Reagent (Factor C, B and the pro-clotting enzyme),
- Conclusion
  - Risk-based approach to identify relevant pyrogens to be covered
  - RPT no longer recommended; Remove or replace with MAT or BET testing
  - rFC/rCR assays encouraged over LAL/TAL assays

Specific considerations-3
-Neurovirulence testing

- Historically, the MNVT used for both the nonclinical assessment and quality control
- Live attenuated viral vaccines: Yellow fever, OPV, Mumps, Other viral vaccines
- Conclusion
  - Nonclinical stage: potential NV be evaluated, in a relevant animal model, until suitable non-animal model become available
  - Experimental animal does not predict residual NV in human, for certain
  - Alternative: Molecular Methods (HTS)

Specific considerations-4
-Potency testing

- The potency of vaccines has traditionally been measured using *in vivo* relative potency assays against reference
- Transition from in vivo to in vitro: understand CQAs of product
  - Concept of consistency
- Validation (one-to-one comparison may not feasible)
- Product content and functionality
- Development of product specific in vitro assay encouraged

Specific consideraations-5
-Specific toxicity

- D, T and Pa, Polysaccharide vaccines conjugated to D T, Oral cholera, Whole cell pertussis vaccines and BCG vaccines
- Specific toxicity and reversion to toxicity
- Conclusion
  - Encourage using in vitro alternatives
  - No redundant testing

Special considerations-6

-Innocuity test

- The immediate **discontinuation** of the inclusion of the innocuity test in all future WHO Recommendations, Guidelines and manuals for biological products published in the Technical Report Series
- The inclusion of this test in previously published WHO Technical Report Series documents be **disregarded**
- List of WHO document mention of innocuity in appendix

In summary

- Review scientific value and relevance of animal test and strategy for QC testing
- Product testing scheme should be science driven
- The guidance is superseding the corresponding quality control recommendations specified in WHO documents published prior to 2025.
- Product developers and manufacturers should develop, validate and implement non-animal-based in vitro approaches to the quality control of biological products

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