

The International Symposium on Regulatory Testing and Animal Welfare: Recommendations on Best Scientific Practices for Biologicals: Safety and Potency Evaluations

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Introduction

This Breakout Group addressed the current best practices and future possibilities for incorporation of refinement, reduction, and replacement (“the 3Rs”) in the safety and potency evaluations of biologicals. Participants in the group (listed at the end of this report) had previously reviewed a number of key background references (also listed at the end of the report) the group leaders had selected before the meeting. They focused on general issues in relation to refinement and considered several more specific issues in relation to particular tests. Participants were asked to consider the following questions as part of their general discussion:

Abnormal Toxicity Test

1. Can others follow the European approach and delete abnormal toxicity testing for routine batch release?
2. Can animals used for potency testing also fulfill requirements for safety testing?

Refinement Procedures

3. How can the introduction of humane endpoints be stimulated?
4. How can humane endpoints be validated?
5. Intracerebral injection
 - a. Is there a need for a Good Practice Guide?
 - b. Is intracerebral injection considered to be painful/distressful, and are there any alternatives?

Replacement Alternatives to Live Animal Potency Testing of Biologicals

6. Can potency testing of vaccines be carried out without a challenge procedure?

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7. Which are the most promising alternatives for the rabies vaccine challenge (National Institutes of Health) test?

Implementation and Harmonization

8. How can the introduction and use of alternatives for routine potency testing be enforced?
9. Is there a need for international validation and harmonization initiatives?

Report on Group Discussion

The Breakout group participants considered the abnormal toxicity test (ATT¹), which was designed to detect any possible contamination in the final lot of product. However, the relevance of the ATT can be questioned as the introduction of good manufacturing practice, good laboratory practice, and new quality control tests should have reduced the potential for contamination. In Europe, the use of the ATT for human vaccines is restricted to the developmental stages. Breakout Group participants believe that the ATT could possibly be eliminated in general for licensed vaccines with a good product history. The ATT is not required at all for veterinary vaccines in Europe. It is required in the United States in a comparable way (e.g., mouse or guinea pig safety test); however, participants believe it may be possible to use the animal potency tests to fulfill the same general safety testing requirements. Mutual recognition of data may be a first step toward the eventual withdrawal of the ATT. There are currently considerable differences in approach between the various countries and regulatory agencies, therefore achieving harmonization would be very difficult. It is likely that the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use will turn its attention to the issue of vaccine tests, albeit not likely in the near future.

Breakout Group participants discussed ways to encourage the introduction and use of humane endpoints for safety and potency testing. In general, they believe that the role of

¹Abbreviation used in this report: ATT, abnormal toxicity test.

regulatory agencies is to disseminate the most recent information and strongly encourage the implementation of earlier, more humane, endpoints. Guidelines should be established for validation of revised methods, including those for previously published results. For the test to be acceptable, at a minimum the following criteria must be met:

- the challenge dose must be infective;
- the reference vaccine must be acceptable (i.e., the test substance dose that results in a 50% reduction in the effect measured [the ED₅₀] value must be within the specified range);
- requirements for linearity and parallelism must be maintained (endpoints must be repeatable); and
- there must be absolutely no reversion in vaccinated animals.

There are currently appropriate, validated earlier endpoints for the rabies, pertussis, tetanus, and diphtheria challenge tests. It is the responsibility of the regulatory agencies to accept humane endpoints and disseminate this information among their stakeholders. Implementation of these endpoints should be strongly encouraged and monitored through observations and inspections. In addition, appropriate earlier endpoints for potency testing should be included in the licensing process during the development of new vaccines. Different observation points (clinical signs, weight loss, body temperature) will apply to different types of products.

Procedures can be refined substantially by ensuring adequate training of employees. Correct and accurate observations and the use of proper techniques (“best practices”) by personnel can further reduce the amount of pain or distress experienced by the animal. Training for injections can be conducted on dead animals with supervision from veterinarians/animal pathologists. Mechanical devices can also assist in performing injections correctly. Of particular concern is the intracerebral injection technique used for some vaccine tests. There is a need for a Good Practice Guide for this procedure, with provision for the consistent use of appropriate anesthesia (e.g., isoflurane), a description of correct technique and use of mechanical devices to achieve correct technique, and, most importantly, appropriate training.

Participants discussed the possibility of reviewing existing dosing guidelines (e.g., for general safety tests) with regulatory agencies, industry partners, and other stakeholders with the view to developing common, appropriate guidelines. In terms of minimizing the use of animals, participants believe that each biological should be reviewed on a case by case basis for safety and potency testing to avoid any unnecessary tests. In addition, for batch tests, sharing of information among manufacturers should be encouraged, perhaps through a symposium facilitated by regulatory agencies. Manufacturers may have a reluctance to share information (to protect proprietary information).

However, many producers have discovered and validated testing methods that could enhance animal welfare, as well as lower the cost of testing, if this information were more widely known.

Participants discussed replacement alternatives for live animal potency testing of certain biologicals. In general, the US Department of Agriculture favors the product-specific antigen quantification approach in contrast to the serological approach, which is favored in Europe for veterinary vaccines (erysipela, tetanus, and other clostridia). Both approaches require validation via correlation to protection tests in target (host) species.

Both approaches should be pursued as appropriate for veterinary vaccines, and the possibilities for human vaccines should continue to be explored and coordinated between the various stakeholders involved at the national level. Sources of funding to carry out trials of new tests also need to be identified. The US Department of Agriculture has a protocol developed to test the validity of an enzyme-linked immunosorbent assay test replacement for *Leptospira* hamster potency tests but currently lacks the funds to conduct it.

Participants considered the rabies vaccine challenge (National Institutes of Health) test. Currently there are both antigen quantification and serological approach alternatives accepted for veterinary rabies vaccines in Europe (by the European Pharmacopoeia); however, they have yet to be implemented. Regulatory agencies could play a significant active role in encouraging the use of these alternatives.

Considerable differences exist in the regulatory test requirements for serial or batch release of biologicals in different countries. The first step is to consider ways to bring about “mutual recognition” of the validity of these different tests. Although international harmonization is desirable, it is also more difficult to achieve. Mutual acceptance by different agencies could be the first step in reducing animal use in regulatory testing; manufacturers would not be required to duplicate animal tests for release in different countries. An international forum for biologicals should be created.

Recommendations

- Consider eliminating the requirement for the abnormal toxicity test for serial release on products with a good history of safety. Use animals in potency testing to also evaluate the safety of the serial.
- Regulatory agencies should disseminate the most recent information and strongly encourage implementation of appropriate earlier endpoints.
- Regulatory agencies should ensure that humane endpoints are being implemented in practice through inspections.
- Training is recommended both to be able to make accurate observations of clinical signs in animals and to ensure that pain and distress for animals are minimized

when performing procedures such as intracerebral injection.

- Develop a Good Practice Guide for the intracerebral injection technique.
- Review existing dosing guidelines to produce commonly accepted guidelines.
- Consider in principle both antigen quantification and serology as potential alternative approaches. However, note that the kind of product being developed will determine the most appropriate method or complementary methods to be used.
- Stakeholders should strive for mutual acceptance of testing guidelines for the safety and potency testing of biologicals. Encourage sharing of information concerning alternatives between manufacturers.
- Identify funding for the development and subsequent validation of new tests with earlier endpoints and improved relevance.
- Create an international forum for biologicals to encourage the harmonization/mutual recognition of safety and potency testing guidelines.

Background References

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