Experience For Acellular Pertussis Batch Release and Implementation of 3Rs

DCVMN:3Rs Experts Working Group Meeting
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Fully-Integrated Vaccine Company
World’s Only Manufacturer of Recombinant Pertussis Vaccines

Production facilities in compliance with WHO and PIC/S GMP

First plant in Thailand with prefilled syringe line
Key principles of the 3Rs

Replace
Avoid using animals whenever possible.

Reduce
When it is impossible to avoid using animals, use the least number possible.

Refine
Use the least number of animals, while ensuring the greatest respect for the animal.
BioNet-Asia Animal Care and Use Committee

- A working group of qualified persons by the Thai law that is in charge of overseeing the safety, respect and dignity of animal subjects involved in scientific research at BioNet-Asia.

- The committee follows Ethical Principles, Thai law, and Guidelines for the care and use of animals for scientific purposes.
Animal Test for Batch Release of aP and TdaP vaccine

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Implementation of 3Rs Concept in BioNet-Asia

✓ Change of Multi-dilution assay to Single Dilution Assay of Mouse Immunogenicity Test for aP Potency.

✓ Deletion of Abnormal Toxicity Test from Batch release test.
European Pharmacopoeia

2.7.16. ASSAY OF PERTUSSIS VACCINE (ACELLULAR)

The assay of acellular pertussis vaccine measures the capacity of the vaccine to induce the formation of specific antibodies in mice or guinea-pigs. Antibody titres for each antigen are determined using a suitable immunochemical method (2.7.1) such as enzyme-linked immunosorbent assay (ELISA).

The assay results can be expressed:

- either as a ratio of the geometric mean titre (GMT) of antibodies produced following administration of the test vaccine to the GMT of antibodies produced following administration of a reference vaccine examined in parallel (relative potency assay);
- or directly as a GMT of antibodies induced by the test vaccine (geometric mean unit assay or GMU assay).

Methods A and B described below are developed by testing multiple dilutions of the test vaccine and the reference vaccine or internal control (see Glossary), to determine which dilutions are suitable. Once the suitable dilutions have been confirmed for a given vaccine, it is recommended, in accordance with 3R principles (Replacement, Reduction, Refinement), to apply a simplified model such as a single dilution for both the test vaccine and the reference vaccine or internal control. Such a model enables the analyst to determine whether the immunogenicity of the test vaccine is satisfactory.
WHO TRS878 Annex2

Immunogenicity test in mice

The immunogenicity test for acellular pertussis vaccine is a standardized assay designed to demonstrate consistent immunogenicity in mice from lot to lot for each antigen in the vaccine. Immunogenicity can be measured as either the geometric-mean amount of antibody produced in mice injected with a test dose of vaccine, or as the minimal dose of each antigen inducing a measurable antibody response in a certain proportion of mice (e.g. the median effective immunizing dose (ED$_{50}$)).

In the first method, a group of mice is injected with a pre-selected dose of vaccine that is within the linear-response region of the dose–response curve (vaccine dose versus antibody production) for a given antigen. After an appropriate length of time, another test dose of vaccine may be required for preparations containing multiple antigens, because of the differential immunogenicity of the antigens in mice. In the second method, groups of mice are injected with serial dilutions of vaccine. After consistency in manufacturing and testing has been demonstrated to the satisfaction of the national control authority, the serial-dose method may be simplified to a single-dose (e.g. ED$_{50}$) for the antigen) assay.

Regardless of test design, the antibody content of test sera is calculated relative to a stabilized reference serum by means of a validated and standardized ELISA.
Multi-dilution assay

- 4 dilutions of aP reference
- 4 dilutions of test vaccine
- Negative control (Normal saline)

Total mice = 144 (16 mice/Gr.)

I.P injection, 0.5 mL 35 Days

Bleed and prepare serum

Indirect ELISA for anti-PT, anti-FHA titer

Analyze relative potency by Parallel line analysis,

Single dilution assay

- 1 dilution of aP reference
- 1 dilution of test vaccine
- Negative control (Normal saline)

Total mice = 48 (16 mice/Gr.)

I.P injection, 0.5 mL 35 Days

Bleed and prepare serum

Indirect ELISA for anti-PT, anti-FHA titer

Analyze relative potency as GMT ratio of aP reference to test sample
3Rs Implementation in collaboration with NRA

- Review Scientific data, New Regulation and Guideline, Historical data of batch releases or perform correlation study of an animal test
- Discuss and Consult with NRA (National Control Laboratory for Biological Products)
- Prepare Summary Report of the Study and Supportive Documents
- Submit Documents for file variation to Thai FDA
Correlation of Multi-Dilution assay and Single Dilution assay of Mouse Immunogenicity Test of Acellular Pertussis vaccine

- Review historical data (50-80 tests) of aP Potency test for batch release and stability study for Boostagen and Pertagen.

- Correlate test results of aP potency calculated from multi-dilution assay and GMT ratio of each vaccine dilution using Paired T test.

- Justify the suitable vaccine dilution to be used in single dilution assay
Proposed Deletion of Abnormal toxicity test

Reasons
• Out of date
• The test to identify potentially harmful batches is highly questionable
• No reliable conclusion can be drawn from the test
• The test cannot be validated according to today’s validation characteristics
• Lack of a sound scientific rationale and justification
• Modern pharmaceutical production and manufacturing facilities are well controlled in compliance with GMP.

Reference: 1. EFPIA’s article: Deletion of test for abnormal toxicity from European pharmacopoeia.
08 December 2017, Strasbourg, France

Suppression of the Test for Abnormal Toxicity from the European Pharmacopoeia

During its 159th plenary session, held in Strasbourg on 21-22 November 2017, the European Pharmacopoeia Commission endorsed the complete suppression of the test for abnormal toxicity from the European Pharmacopoeia (Ph. Eur.).

As part of this exercise, 49 monographs revised to remove the test for abnormal toxicity adopted by the Commission; notably, these included 36 monographs on vaccines for human use. In addition, as the general chapter Abnormal Toxicity (2.6.9) will no longer be referred to in any monograph, it will subsequently be rendered obsolete and will also be deleted from Ph. Eur.

Deletion of the abnormal toxicity test was issued in European Pharmacopoeia 9.8 and becomes effective on 01 January 2019
Thanks to My Research & Development, and Quality Control Team
Thank You ขอบคุณครับ

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