BioNet
sharing vaccine expertise

Experience For Acellular Pertussis Batch Release and Implementation of 3Rs

DCVMN:3Rs Experts Working Group Meeting

02 December 2019, Bangkok, Thailand

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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

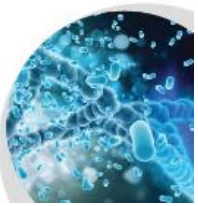
BNAACUC

BioNet-Asia Animal Care and Use Committee

- A working group of qualified person 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

Animal Test
aP Potency (Mouse Immunogenicity Test)
Diphtheria Potency (Guinea Pig-VERO cell assay)
Tetanus Potency (mice challenge)
Specific toxicity (Tetanus and Diphtheria)
Abnormal Toxicity



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.

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

European Pharmacopoeia

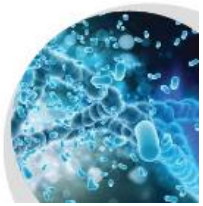
2.7.16. ASSAY OF PERTUSSIS VACCINE (ACELLULAR)

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.



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

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

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Discuss and Consult with NRA
(**National Control Laboratory for Biological Products)**

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Prepare Summary Report of the Study and Supportive Documents

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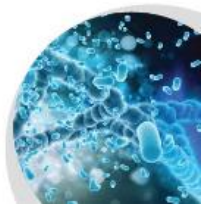
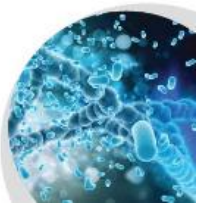
Submit Documents for file variation to Thai FDA



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.
2. Garbe et al, Historical Data Analyses and Scientific Knowledge Suggest Complete Removal of the Abnormal toxicity Test as Quality Control Test J. Pharm. Sci. 103:3349-3355, 2014

08 December 2017, Strasbourg, France

Suppression of the Test for Abnormal Toxicity from the Europ Pharmacopoeia

During its 159th plenary session, held in Strasbourg on 21-22 November 2017, the Europ Pharmacopoeia Commission endorsed the complete suppression of the test for abno 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 hu use. In addition, as the general chapter Abnormal Toxicity (2.6.9) will no longer be refere 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

DELETED TEXTS

*The following texts are deleted as of **1 July 2019**.*

MONOGRAPHS

Sutures for veterinary use

Polyamide 6 suture, sterile, in distributor for veterinary use (0609)

Polyamide 6/6 suture, sterile, in distributor for veterinary use (0610)

Monographs

Dihydroergotamine tartrate (0600)

*The following texts are deleted as of **1 April 2019**.*

MONOGRAPHS

Monographs

Chlorpropamide (1087)

Oxprenolol hydrochloride (0628)

Water, highly purified (1927)

*The following texts are deleted as of **1 January 2019**.*

GENERAL CHAPTERS

2.6.9. Abnormal toxicity

Thanks to My Research & Development, and Quality Control Team

Thank You ขอบคุณครับ

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BioNet
sharing vaccine expertise