

# Alternative testing methods for pertussis vaccine



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# Scope

In this session I will discuss the progress and achievements of 3Rs in the development of alternative test methods involved in the testing of Pertussis component.

# Kendrick or Mouse Protection Test (MPT)

The current pertussis potency test for whole-cell vaccine is based on the intracerebral mouse protection test as described by Kendrick (1947).

The predictive value of this test for the protective activity of the vaccine in man was investigated by the Medical Research Council (1956).

The Kendrick test is an assay designed to estimate the potency of pertussis containing vaccines on the basis of their ability to protect mice against intra-cerebral challenge with virulent *Bordetella pertussis*.

# Kendrick or Mouse Protection Test (MPT)

## +ve features

Developed in 1947: 70 years experience

Extensive data base for: development& routine

release of wP vaccines, stability testing, etc.

A functional test

Clinical efficacy of the vaccines passing the test

# Kendrick or Mouse Protection Test (MPT)

## -ve features

Low precision and success rate

Limited information on the vaccine characteristics

Biohazard (virulent *B. pertussis*)

Expensive test

Huge no. of animals

High severity level animals

Animal no/test about > 180, including virulence testing challenge culture

# Alternatives to MPT

## Model I: MPT using humane endpoints

Using (early) clinical signs to reduce period of severe suffering. Clinical Signs are indicative for death within observation period (Hendriksen *et al.*, 1999)

## Model II: The intranasal challenge test

Used for R&D purposes, high dose of infection, no signs of pertussis (van der Ark *et al.* Expert Rev Vaccines 2012). Predicts efficacy in children for both whole cell as well as acellular pertussis vaccines (Mills *et al.* Dev. Biol. Stand. 1998), but for acellular pertussis vaccine could not be confirmed in international collaborative study (Xing *et al.*, Vaccine 2007)

# Alternatives to MPT

## Model III: The Nitric Oxide induction assay

Induction of nitric oxide in murine macrophages after stimulation with whole cell pertussis vaccine. Validation is needed (Canthaboo *et al.*, *Dev. Biol. Stand.* 1999).

## Model IV: The pertussis serological potency test (PSPT)

Alternative to the Kendrick test, less variable results and distress to the animals is less (Von Hunolstein *et al.*, *Pharmeuropa Bio* 2008)

Release test for acellular pertussis vaccine, but no direct correlation with protection in humans (van der Ark *et al.*, *Expert Rev Vaccines* 2012).

# PERTUSSIS SEROLOGICAL POTENCY TEST

## SUMMARY OF ACTIVITIES

**1994: Van der Ark *et al.*: Development of Pertussis Serological Potency test** (Biologicals 22, 233-242).

**2000: Van der Ark *et al.*: The Pertussis Serological Potency test.**

Collaborative study to evaluate replacement of the Mouse Protection Test (Biologicals 28, 105-118).

Study partners: 1. Instituto Nacional de Biologica, Argentina

2. National Public Health Institute, Finland

3. Serum Institute of India, India

4. Chiron-Behring, Germany

5. RIVM (organizer & coordinator)

**2008: Von Hunolstein *et al.*: Evaluation of two serological methods for** potency testing of whole cell pertussis vaccines (Pharmeuropa Bio 1, 7-18).

# PERTUSSIS SEROLOGICAL POTENCY TEST

## SUMMARY OF ACTIVITIES

No direct one-to-one correlation was found between MPT and PSPT. However, potency ranking of wP vaccine batches was similar in both tests. Moreover, the PSPT was able to discriminate between compliant and altered batches of wP vaccines.

Therefore, it was proposed to use the PSPT as part of a consistency testing approach, that includes a second, preferably qualitative assay. This extends the number of quality parameters tested, thereby increasing the chance of broad regulatory acceptance.

# Specific toxicity test

## Mouse weight gain test (MWGT)

The MWGT is considered as a general, non-specific test measuring overall toxicity of pertussis whole cell vaccine, since a number of *B. pertussis* toxins may induce weight loss in mice.

Correlation of results of the MWGT with adverse reactions in children has been reported. It is a test used to assess the toxicity of whole cell pertussis containing vaccines, and it is based on the ability of certain toxins or components from *B. pertussis* to cause weight loss in young mice.

# Other tests for monitoring pertussis toxicity

**Not regulatory tests for lot release** of the whole cell pertussis vaccine. However, they may be used for in-house monitoring of the products consistency or for validation of the inactivation procedures.

## **Chinese Hamster Ovary (CHO) cells assay for pertussis toxin**

The CHO cell assay is an *in vitro* assay to assess the active pertussis toxin (PT) content of pertussis containing vaccines on the basis of the morphological changes to Chinese Hamster Ovary (CHO) cells in the presence of active PT. In this test, the CHO cells are treated with different dilutions of PT reference /or test vaccine. After incubation, the degree of clustering of the cells is observed and scored under an inverted microscope.

The highest dilution of the test vaccine showing total cell clustering represents the titre. The amount of active PT in the test sample can be semi-quantified against a reference preparation of known concentration.

## 3Rs approaches for Pertussis

- Currently testing of pertussis vaccines largely based on challenge based procedures.
- SII is working on various alternatives:
  - Histamine challenge sensitization method
  - Flow cytometry based methods for quantifying pertussis toxin based effects on leukocytes

# Perspective

SIPL always aimed for alternatives. Have been partner to various international collaborative studies aimed at 3Rs on Diphtheria, Tetanus, and Pertussis since 1999.

<b>Tetanus Vaccine</b>	<b>BSP035</b>	<b>EDQM: Invitro methods for alternatives to challenge test of tetanus toxoid.</b>
<b>Diphtheria Verocell assay</b>	BSP034	EDQM: Verocell assay as alternative to diphtheria potency test.
<b>Pertussis (PSPT)</b>	Serological assay	Humane Endpoints for Lethal Parameters (HELP) funded by ECVAM.

- Regulatory acceptance: (Non animal methods are being accepted for release of vaccines for example, hepatitis B vaccine, glycoconjugate vaccines. Similar approach may be followed for rabies)
- Mechanisms to introduce such recommendations in regulatory documents, pharmacopeias
- Harmonization of regulatory requirements: Important for global supplier like us.

# Reference:

## WHOLE-CELL PERTUSSIS VACCINES AND DEVELOPMENT OF ALTERNATIVE INVIVO AND IN VITRO POTENCY TESTS

Presentation by  
INTRAVAC

DCVMN Workshop, May 2018 Hyderabad

# Thank You



# Histamine sensitization assay

There are two methods that are currently used for histamine sensitization assay.

1. Based on temperature measurement (Temperature method) &
2. Based on histamine-sensitizing death.

## ***Histamine sensitization assay (Temperature method)***

Mice inoculated with pertussis toxin become highly sensitive to a histamine challenge. The effects include reduction in body temperature and in the severe cases death. The reduction in body temperature occurs within 30 minutes after histamine challenge, but in the non-lethal situations it returns to normal levels after 30 minutes. Therefore, reduction in body temperature 30 minutes following histamine challenge is directly proportional to the dose of active PT present in the vaccine. This method is highly sensitive, it can detect levels of PT activity that do not induce lethal effects following histamine challenge.

## Histamine sensitization assay (Lethal end-point method)

An assay to assess the active pertussis toxin (PT) content of pertussis containing vaccines on the basis of the histamine sensitizing effect of active PT on mice.

Pertussis toxin increases the sensitivity of mice to histamine. The exact mode of action is not yet fully understood. Even when small amounts of active PT are present in a vaccine, mice will become vulnerable to challenge with histamine, resulting in anaphylactic shock and inevitable death.

The amount of histamine sensitization factor (HSF) activity in a vaccine can be quantified in a parallel-line assay in comparison with a reference vaccine. In this assay the reference and test vaccine doses, which induce a histamine sensitization in 50% of the animals, as measured by death after challenge with histamine, are compared and a relative HSF activity is calculated for the vaccine.

Different mouse strains may show different sensitivity to the test, laboratories are recommended to set up their own experimental conditions.