

3Rs methods applicable to control the quality of Diphtheria and Tetanus vaccine components



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Scope

Vaccines are of biological origin and have the potential to vary from batch to batch. Consequently, vaccines are tested for batch-to-batch consistency and many of these tests involve animals i.e. it is at the expense of large numbers of animals that are used in quality control tests before vaccines are released onto the market.

In this session, I will discuss the progress and achievements of 3Rs in the development of alternative test methods involved in the testing of D and T components.

Importance of 3Rs in the Field of Vaccinology

The use of "alternative" methods are generally concerned with the Principles of the 3Rs - Replacement, Reduction, and Refinement of animal testing, first proposed by the scientists William Russell and Rex Burch in their book 'The Principles of Humane Experimental Technique' (1959).

The animal models have several limitations in respect of their relevance, reliability, costs, ethical acceptability and also the process is laborious and time consuming. Moreover, the injection of virulent material or manipulation of contaminated animals exposes technicians to additional risks.

Importance of 3Rs in the Field of Vaccinology

The potency tests like • challenge assays • toxin neutralization assays • serological assays etc. are mainly used for quality control evaluation of vaccines. Mostly potency assays based on an immunization-challenge procedure in laboratory animals are still being used in vaccine research and routine lot-release testing.

These potency tests are multi-dose models that include a challenge procedure with virulent micro-organisms. As a result, animals suffer substantial pain and distress during the testing period.

The quality control evaluations of vaccines require high frequency of tests with large number of laboratory animals.

Testing for Diphtheria Vaccines

Potency

1. Potency in guinea pigs by lethal challenge: The challenge potency test for diphtheria vaccine (adsorbed) is determined by comparing the dose of the vaccine to that of a reference preparation required to protect guinea pigs from lethal toxin challenge. It is a multi dilution assay.

2. Single dilution test : It is a one dilution assay and is performed: 1.when the potency of the test vaccine consistently and significantly exceeds minimum requirements i.e. the potency of the test vaccine is significantly greater than the minimum requirement per human dose for the product under test and 2. when parallelism between test and reference vaccine has been demonstrated over time.

Testing for Diphtheria Vaccines

3. Potency in guinea pigs by serology :

Serology is an alternative procedure to the guinea pig challenge method, antibody responses to diphtheria toxoid induced in guinea pigs after 5 to 6 weeks are compared relative to the antibody response induced by the reference vaccine.

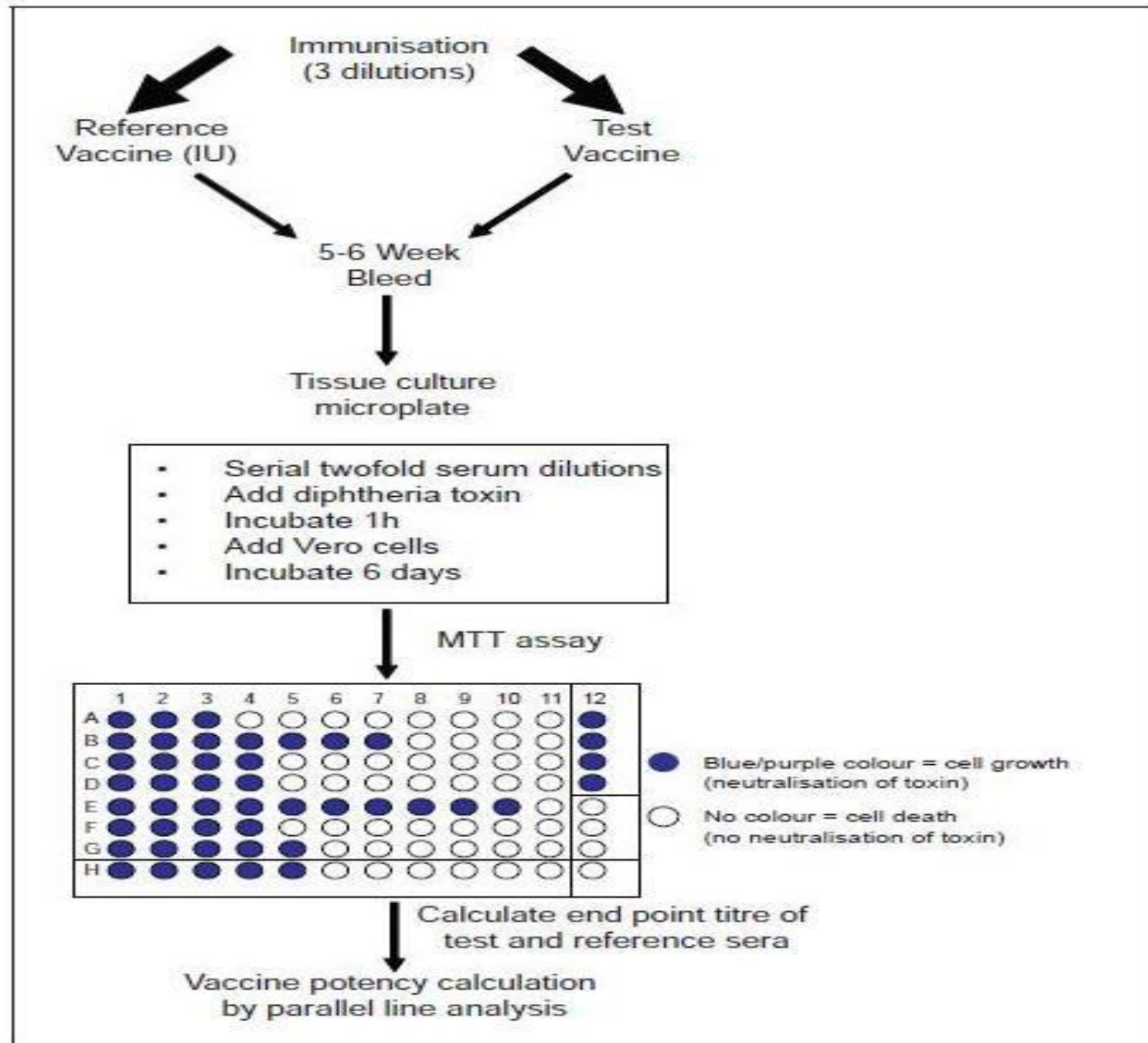
WHO recommends that functional end-point assays such as serology using Vero cells can be used as an alternative procedure to the challenge tests in guinea pigs for determination of diphtheria vaccine potency.

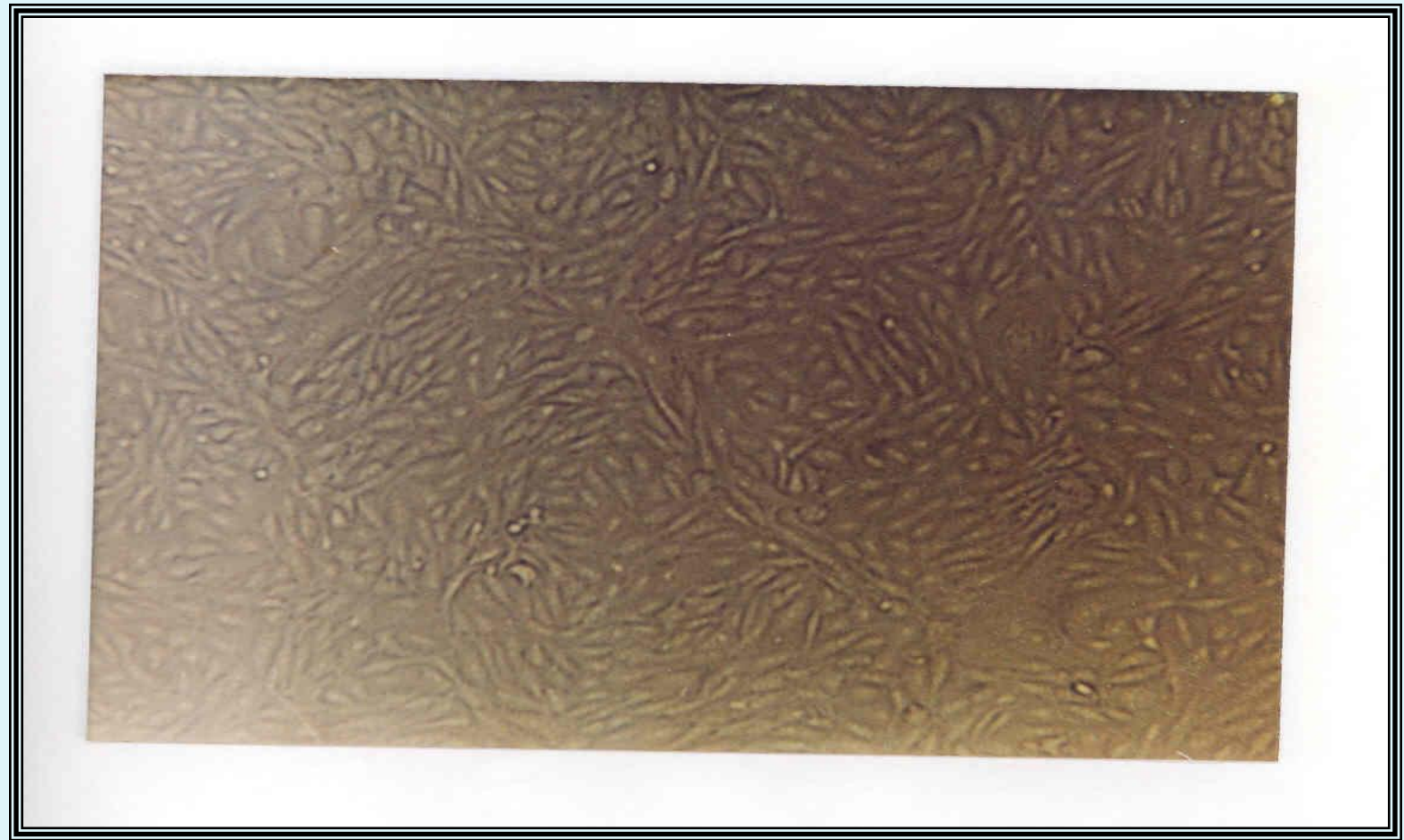
4. Potency in mouse by Vero cell assay :

The mouse potency assay involves the detection of functional antibodies against diphtheria toxoid induced in mice by observing neutralizing effect of sera on diphtheria toxin in a Vero cell culture model.

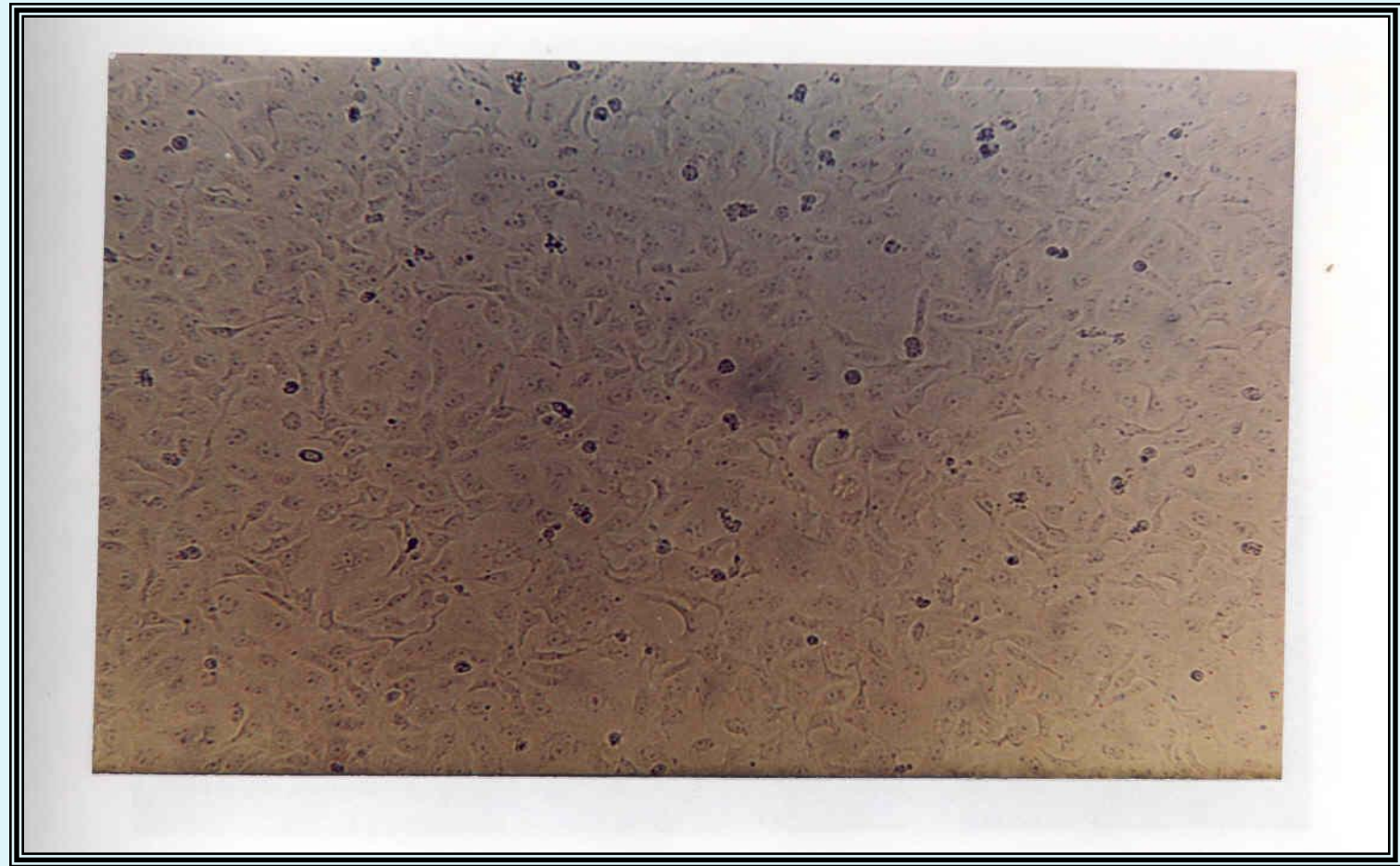
(mice are not sensitive to diphtheria toxin)

Schematic diagram of the Vero cell assay for potency testing of diphtheria vaccine

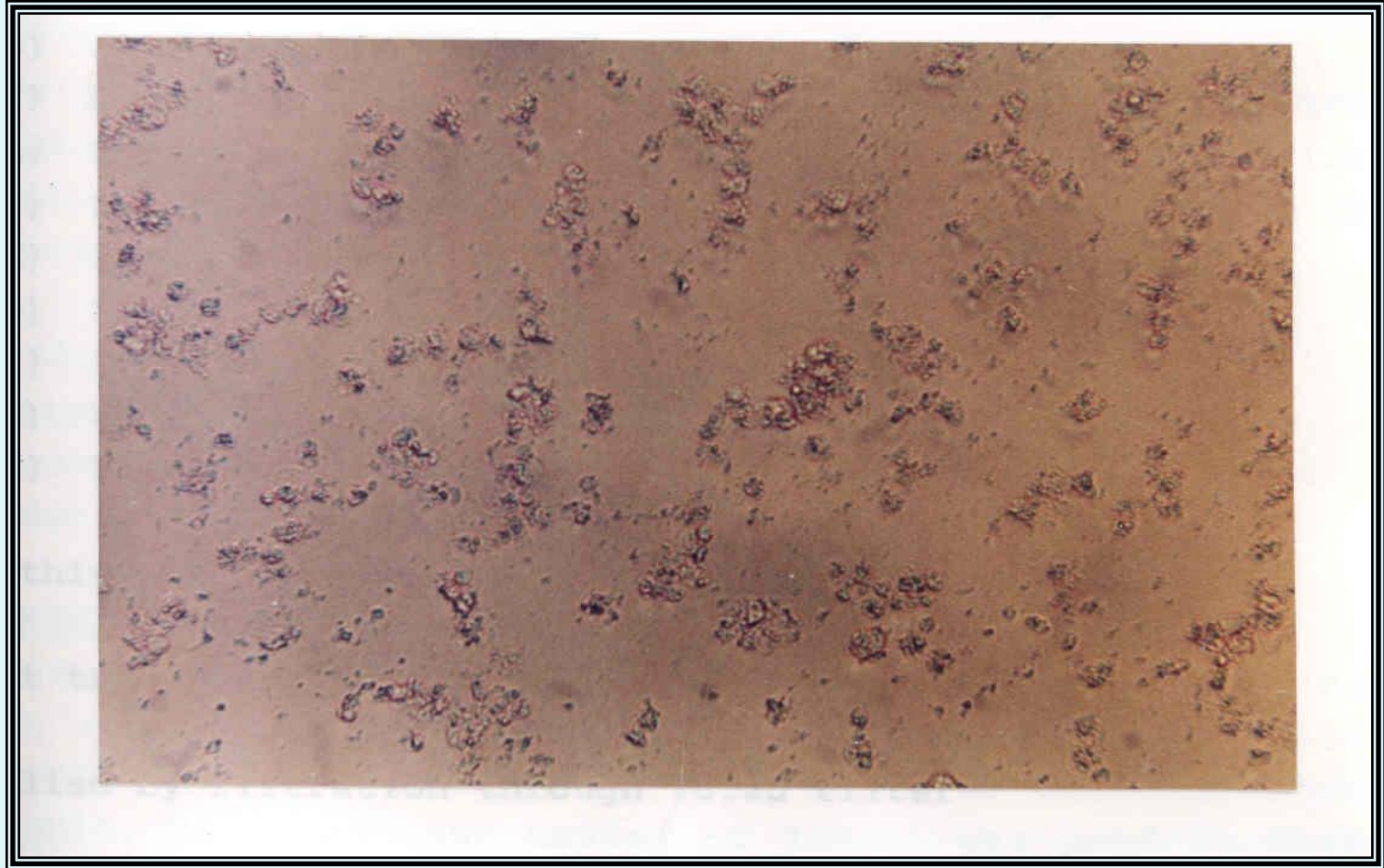




EFFECT OF DIPHTHERIA TOXIN ON VERO CELLS – HEALTHY CELLS



**EFFECT OF DIPHTHERIA TOXIN ON VERO CELLS – PARTIALLY
AFFECTED CELLS**



**EFFECT OF DIPHTHERIA TOXIN ON VERO CELLS – IRREVERSIBLE
DEATH/METABOLIC INHIBITION**

Testing for Diphtheria Vaccines

5. In vivo toxin neutralization test:

The *in vivo* toxin neutralization test (TNT) can be performed on the depilated skin of guinea pigs owing to the ability of diphtheria toxin to cause an erythematic reaction when injected intradermally.

Specific Toxicity

In vivo test for absence of toxin and absence of reversion to toxicity in guinea pigs:

This in vivo assay remains the method of choice for routine testing or validation of production processes.

Vero cell test for absence of toxin and absence of reversion to toxicity: A Vero cell culture system may also be used as an alternative to in vivo tests for specific toxicity and reversion to toxicity.

Testing for Tetanus Vaccines

Potency

1. Potency in guinea pigs and mice by challenge (lethal and paralysis):

The challenge potency test for tetanus vaccine (adsorbed) is determined by comparing the dose of the vaccine to that of a reference preparation required to protect guinea pigs or mice from either a lethal or paralytic toxin challenge.

2. Single dilution test : It is a one dilution assay and is performed (i) when the potency of the test vaccine consistently and significantly exceeds minimum requirements i.e. the potency of the test vaccine is significantly greater than the minimum requirement per human dose for the product under test and (ii) when parallelism between test and reference vaccine has been demonstrated over time.

Testing for Tetanus Vaccines

3. Potency in guinea pigs by serology :

Serology is an alternative procedure to the guinea pig challenge method, antibody responses to tetanus toxoid induced in guinea pigs after 5 to 6 weeks are compared relative to the antibody response induced by a reference vaccine.

WHO recommends that Enzyme Linked Immunosorbent Assay (ELISA) can be used with serology assays to determine the potency of tetanus vaccine for routine lot release after its validation against the challenge assay or the toxin neutralization test.

4. Titration of immune sera by ToBI

WHO recommends that Toxin Binding Assay (ToBI) can be used with serology assays to determine the potency of tetanus vaccine for routine lot release after its validation against the challenge assay or the toxin neutralization test.

Testing for Tetanus Vaccines

5. Potency in mice by serology (ToBI) : Potency test for routine lot release can be performed by immunizing mice as well as guinea pigs with appropriate dilutions of the calibrated reference preparation and the test vaccine. Titration of immune sera may be performed in vitro by ToBI test.

Specific Toxicity

In vivo test for absence of toxin and reversion to toxicity in guinea pigs: The purpose of the specific toxicity test for tetanus toxin is to confirm freedom from residual toxin and reversion to toxicity in final bulk vaccines and/or bulk purified toxoid. The in vivo assay remains the method of choice for routine testing or validation of production processes.

Implementation of 3Rs in quality control testing of vaccines at Serum Institute

- ▶ Serum Institute of India Pvt. Ltd. (SI IPL)-PUNE is India's largest manufacturer of vaccines and other biotech products.
- ▶ SI IPL vaccines produces several bacterial, viral and recombinant vaccines. SI also manufactures combination and multivalent vaccines.
- ▶ SI IPL is committed to the development, introduction, validation, and implementation of 3Rs (Refinement, Reduction, and Replacement) and consistency based approaches.
- ▶ India Pharmacopoeia has always been supportive and receptive to such initiatives.

Serological testing and DPT vaccines: 3Rs Opportunity

- Potency testing of conventional vaccines such as DTP group of vaccines involves challenge methods. The methods require large numbers of animals and induce substantial levels of suffering.
- One of the most feasible alternatives is serology. Serology allows quantitative (antibody titers) instead of qualitative endpoints (death or survival) and also a significant reduction in the number of animals used.
- SII committed itself for development of such serology based alternatives.

DTP GROUP OF VACCINE (Serological assays): Replacement

CONVENTIONAL METHOD

Lethal Challenge test

Animals used : Guinea
pigs / Mice



ASSAYS FOR 3R APPROACH

- Vero cell assay (Potency of Diphtheria component)
- T-ELISA (Potency of Tetanus Component)

Serological testing and DPT vaccines: 3Rs opportunity (Reduction)

- ▶ The serological are multi-dilution assays and thus imply still the use of great number of animals.
- ▶ Single dilution assays were considered a reduction approach once production consistency has been established.
- ▶ Implementation requires an extensive and rigorous comparison with multi-dilution assay.
- ▶ SIPL team committed itself on the same and data on large number of batches was submitted to National control laboratory for approval.
- ▶ SIPL got the approval for these assays from the **NCL**, **NRA** and subsequently from **WHO**.
- ▶ SIPL was successful in implementation of such assays for routine testing and control.

Single dilution Vero cell and T-ELISA Assay: Regulatory acceptance

Steps in process of test development	<u>Timelines</u> (Diphtheria)	<u>Timelines</u> (Tetanus)
Development of assay	June 2003	2004
Submission of proposal to National Control Laboratory	October 2005	October 2006
Final acceptance by National Control Laboratory	March 2006	August 2007
Approval obtained	1 in 10 lots needs to be tested in challenge test. April 2017 - Approval for 1 in 25 lots to be tested or once in six months which ever is earlier.	

Implementation of 3Rs approaches at SIIPL and annual animal savings

Potency	Method	Animal model	Duration of Test (days)	Number of animals per lot
Tetanus Potency	Lethal challenge assay	Guinea Pigs	33	116
Diphtheria potency	Lethal challenge assay	Guinea Pigs	33	116
Total G. Pigs				232

Tetanus and Diphtheria potency together In case of DT, DTP and combined vaccines	Method	Animal	Duration (days)	No. of animals	% reduction in animal consumption
	T-ELISA & Vero cell assay	Guinea pigs	44 & 49	Std. T – 10 Std. D – 10 Test Vaccine-10 Total = 30	~ 85%

Implementation of 3Rs approaches at SIIPL and annual animal savings

Potency	Method	Animal model	Duration of Test (days)	Number of animals per lot*
Tetanus Potency	Lethal challenge test	Guinea Pigs	33	116
Tetanus Potency	*Paralytic challenge test	Mice	33	126
*NCL's approval obtained to perform Paralytic Challenge Assay in Mice in 2017 Total G. Pigs Replaced with Mice				116

Perspective

SIII always aimed for alternatives. Have been partner to various international collaborative studies aimed at 3Rs on Diphtheria, tetanus, and pertussis since 1999.

Tetanus Vaccine	BSP035	EDQM: Invitro methods for alternatives to challenge test of tetanus toxoid.
Diphtheria Verocell assay	BSP034	EDQM: Verocell assay as alternative to diphtheria potency test.
Pertussis (PsPT)	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

**WHO Manual for Quality Control of Diphtheria, Tetanus
and
Pertussis Vaccines**

WHO/IVB/11.11

Thank You

