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Implementation of 3Rs in vaccine testing at BBIL

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INTRODUCTION

The people who work in laboratories – scientists, vets, animal carers – are human beings like everyone else and have no desire to mistreat animals.

The guiding principles underpinning the humane use of animals in scientific research are called the 3Rs. Any researcher planning to use animals in their research must first show why there is no alternative and what will be done to minimize numbers and suffering for animals, through any of the following methods:

- Replace the use of animals with alternative techniques.
- Reduce the number of animals used to a minimum.
- Refine the way experiments are carried out, to make sure animals suffer as little as possible.

SCOPE

Current guidelines and Pharmacopoeias encourage stakeholders and authorities to initiate, support, accept and implement the use of 3Rs testing approaches in vaccine testing for human and veterinary use.

In view of the necessity of implementation of 3Rs in QC testing of vaccines , the scope of this presentation defines the current and prospective status of 3Rs especially in Diphtheria and Tetanus components potency testing at BBIL:

- 3Rs **to be implemented** at BBIL
- 3Rs **in-process of implementation** at BBIL
- 3Rs **already implemented** at BBIL

Assays available for Diphtheria Potency as per Pharmacopoeia and WHO guidelines

- Intradermal Challenge Assay
- Antibody induction method
- Lethal challenge method
- Any other validated serological assay in guinea pig or mice as approved by national regulatory authorities.

Summary of the three Pharmacopoeial assays as mentioned above:

Test description	Animal used	No of animals used	Route of immunization	Route of challenge	Duration of assay
Intradermal Challenge Assay	White guinea pigs	Sufficient for a valid assay	Subcutaneous	Intradermal	30
Lethal challenge method	Guinea pigs	112	Subcutaneous	Subcutaneous	33
Antibody induction method	Guinea pigs	12	Subcutaneous	Intracutaneous	44-51

Assays available for Diphtheria Potency as per Pharmacopoeia and WHO guidelines

Single Dilution Assay:

- Single dilution test for Lethal Challenge Assay: When a single dilution assay is performed, the potency of the test vaccine should be demonstrated to be significantly greater than the minimum requirement per human dose for the product under test. The single dilution assay requires previous demonstration of the following parameters:
- the potency of the test vaccine consistently and significantly exceeds minimum requirements.
- a significant regression has been demonstrated for the test vaccine over time
- parallelism between test and reference vaccine has been demonstrated over time.

3Rs to be implemented at BBIL

Serological methods intended to be adoptd after evaluation at BBIL:

A: Antibody induction method as per Pharmacopoeia

- Inject subcutaneously on two occasions 28 days apart, 1ml of 1/50 the dose diluted to 1 ml with saline solution.
- Number of animals: 10 guinea pigs.
- Bleeding 2-3 weeks after booster.
- The potency of the antitoxin is determined by comparing the dose necessary to protect guinea pigs or rabbits against the erythrogenic effect of a fixed dose of standard preparation of diphtheria toxin.
- The geometric mean of the antitoxin contents shall be not less than 2.0 units per ml with reference to diphtheria antitoxin standard.

3Rs to be implemented at BBIL

B. Diphtheria Potency as per WHO manual:

Immunization of animals followed by bleeding after 35-42 days and titration of antibodies by using an appropriate kit/ method mentioned in WHO manual.

S.No	Test Parameter details	Number of animals (Guinea pig)	Duration of testing (days)
1	Lethal Challenge assay	112	33
2	Proposed Assay	62	47

(Reduction of animals used: 50)

(Refinement: Non exposure of animals to after effects of toxin inoculation)

3Rs to be implemented at BBIL

Brief of testing procedure:

- Route of immunization: S/C 1 ml of sample, dilutions eg. 1:5 to 1:45 for sample and standard.
- Positive control guinea pig antiserum NIBSC 98/572.
- Bleeding after 5 to 6 weeks.
- Sera Titration using ELISA
- Final Relative antibody titre: $\frac{\text{Relative estimate test sample X Dilution factor of sample}}{\text{Dilution factor of positive control antiserum}}$

  Log response of the sample is plotted against log of vaccine dose immunized in animals.
- Potency of the test vaccine is calculated with respect to the reference vaccine preparation, calibrated in IU by comparing both the dose response curves in a parallel line assay.
- Assay shall meet the validity criteria as per WHO guidelines

Assays available for Tetanus Potency as per Pharmacopoeia and WHO Guidelines

- Test in Guinea pigs or mice by Lethal Challenge Assay
- Antibody induction method in Guinea pigs
- Determination of antibodies in guinea pigs by ELISA/ ToBI
- Any other validated serological assay as approved by national regulatory authorities.

Summary of the assays as mentioned above:

Test description	Animal used	No of animals used	Route of immunization	Route of challenge	Duration of assay (days)
Lethal Challenge Assay	Guinea pigs	116	Subcutaneous	Subcutaneous	33
Antibody induction method	Guinea pigs/ mice	9, 12	Subcutaneous	Subcutaneous	46
Lethal challenge method	Mice	120	Subcutaneous	Subcutaneous	33
Determination of antibodies in guinea pigs by ELISA/ ToBI	Guinea pigs	38	Subcutaneous	NA	46

Assays available for Tetanus Potency as per Pharmacopoeia and WHO Guidelines

- **Single dilution Assay:**

  After establishing consistency/ stability of product by any of the multiple dilution assay, a single dilution assay based on serological methods or other principles may be used.

- The assay involves the selection of dose of the reference vaccine, expressed as a fraction of 40 IU in guinea pigs/ 60 IU in mice (i.e of the minimum potency of single human dose), that elicits a minimal protective effect, and comparing its effect with the response elicited by the same fraction of a human dose of the test vaccine.

- If the response to the test vaccine is significantly greater than the response of reference vaccine ($P<0.05$), the potency of the test vaccine is satisfactory.

3Rs to be implemented at BBIL

Serological methods for Tetanus Potency as per WHO manual intended to be adopted after evaluation at BBIL:

Immunization of animals followed by bleeding after 28-35 days and titration of antibodies by using ToBI as per WHO Manual.

S.No	Test Parameter details	Number of animals (mice)	Duration of testing (days)
1	Lethal Challenge assay	120	33
2	Proposed Assay	60	40

(Reduction of animals used per assay: 60)

3Rs to be implemented at BBIL

Brief of testing procedure:

- Route of immunization: Subcutaneous 0.5 ml of sample, dilutions eg. 2-13 IU/dose for sample and standard into right groin fold of mice.
- 4 control mouse sera with known score (e.g. 0, 2, 4, 7) or suitable mouse antiserum.
- Negative control serum, from non-immunised mice/ injected with saline.
- Bleeding after 4 to 5 weeks.
- Serology by ToBI as per WHO manual.
- Toxin solution containing the test dose of 0.1 Lf/ml for toxin binding assay.
- OD50 value for each plate is calculated by applying the following formula:

$$\frac{[\text{mean absorbance value of positive Control} + \text{mean absorbance value of negative control}]}{2}$$

- The potency of the test vaccine is calculated with respect to the reference preparation, calibrated in IU, using parallel line regression analysis .
- Assay should be valid as per WHO guidelines.

3Rs to be implemented at BBIL

- Enrichment as a measure for refinement and providing better environment for animals during testing :

- Soft music for animals
- Small playing balls in mice cages, exhausted tissue paper rolls shall be used as enrichment.
- Provision for appropriately sized pipes for guinea pigs hiding during day light in cages.

3Rs in-process of implementation at BBIL

- **Removal of Abnormal Toxicity and Pyrogen test as per IPC meeting held in April 2019 at CRI Kasuali, for the under mentioned vaccines:**
  - Hepatitis B (rDNA) vaccine
  - Rabies vaccine, Human
  - Typhoid Vi Conjugate vaccine (Typbar-TCV)
  - Japanese Encephalitis vaccine

  (Data ready for submission to NCL/ NRA).
- Removal of **Abnormal Toxicity** from the routine lot release of typhoid polysaccharide vaccine.
- Hepatitis B (rDNA) vaccine:

  Complete waiver off for *in-vivo* potency and release of batches on the basis of *in-vitro* **potency assay** as mentioned in Pharmacopoeia.

3Rs already implemented at BBIL

- Humane end points implemented for lethal challenge method in Tetanus potency assays performed at BBIL in DPT and its combination vaccines . i.e T3 (paralysis of the toxin injected hind leg, which does not function for walking) is considered as end point instead of death.

- NIH potency assays for human rabies vaccine were performed in replicates of two at BBIL. Applied for waiver off for 2nd potency assay (NIH) for rabies after evaluation and submission of data in the past years and got approval from National Control Laboratory for the same.

  (Reduction of animals per batch = 160 mice).

- Waiver off for Hep-B In-vivo potency for every lot from NCL, Instead Potency assay to be performed on every 4 th batch manufactured for Hepatitis B vaccine.

  (Reduction of animals per batch except 4th batch = 180 mice).

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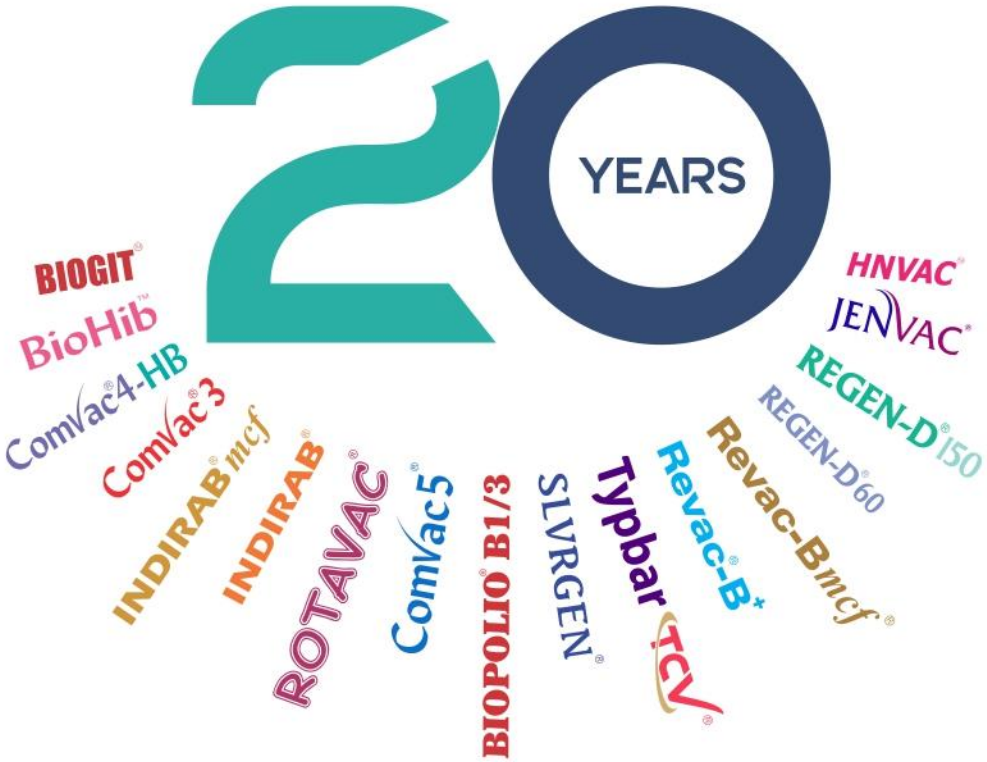
THANK YOU



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