

Considerations on alternative testing for Rabies Vaccine



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Regulatory requirement for quality control of rabies vaccines

- ▶ Regulatory guidelines worldwide outline tests to be performed at different stages of vaccine production to monitor the quality, safety and efficacy of vaccines as all the processes require to be strictly controlled to ensure consistency of production. Tests for safety, inactivation, pyrogenicity and potency are being performed in animals.
- ▶ A very large number of animals are required for these tests and in particular for potency the maximum number of animals (at least 120 mice) per batch are needed.
- ▶ In this session I will discuss the progress and achievements of 3Rs in the development of alternative test methods involved in the testing of Rabies Vaccine.

Three Rs alternatives –Regulatory policies

- ▶ **Ph. Eur.** Clearly states its commitment to reduction of animal use and encourage to seek alternative (in vitro) procedures.
- ▶ **OMCLs** are encouraged to evaluate their testing procedures and to identify candidates for which *in vivo* testing can be reduced without compromising product quality and safety.
- ▶ **CBER** encourages the manufacturers to replace animal based potency tests by submitting a supplement to the license detailing the new test with validated test protocol and sufficient data in support for acceptance.
- ▶ **WHO-** had also expressed its view of replacing animal potency test with antigen quantification procedure which is yet to be finalized.

Potency Testing – NIH Test

- ▶ Standard method - multi dilution vaccination - challenge test in mice performed on each lot and is also used for stability testing.
- ▶ In some countries average of 2 tests is taken.
- ▶ Large number of mice are consumed
- ▶ Inflict great suffering in mice
- ▶ Test is time consuming (28 Days)
- ▶ Risk of infection to lab staff
- ▶ Test results are highly variable (25% to 400%)
- ▶ Unable to demonstrate correlation between *in vitro* and *in vivo* data

Possibilities for Reduction of animals

- ▶ Single dilution test- can be used as a screening test once experience has been gained in the lab.
- ▶ Reduction in number of animals per group depending upon the type of assay and data available
- ▶ Verification of challenge dose : Potency testing of several batches to be performed in parallel with only one test for verification of challenge and reference.
- ▶ Frequency of testing: Only one potency test to be performed, second test does not contribute to the test precision.

Possibilities for Refinement

- ▶ Use of anaesthetics in order to reduce the pain and distress caused by intra cerebral injection
- ▶ Intra cerebral injection technique : Inoculation only by trained analysts.
- ▶ Criteria for evaluation of potency test: Only non-lethal endpoints should be used as a criteria for test evaluation.

Possibilities for Replacement

There is an urgent need for replacement of NIH Test and its variants:

1. Serological alternatives:

Various serological tests like

- (i) RFFIT- Rapid Fluorescent Focus Inhibition Test and
- (ii) FAVN – Fluorescent Antibody Virus Neutralization Test

allow the quantification of rabies virus neutralizing antibodies in the serum of immunized animals. In these tests serially diluted test sera are pre incubated with a given amount of rabies virus prior to inoculation on a sensitive cell culture i.e. BHK -21 cells. After incubation, the quantity of un neutralized rabies virus is revealed by immuno fluorescence.

- ▶ (These tests have their own advantages and disadvantages)

Possibilities for Replacement

2. Alternatives based on antigen quantification:

- (i) Single Radial Immuno Diffusion Test (SRID): In this test the rabies virus contained in the vaccine is split by means of a detergent and the concentration of free glycoprotein is then estimated by measurement of diffusion zones in a gel containing antibody specific for the glycoprotein. It is rapid, inexpensive and does not require any special equipment.
- (ii) ELISA: Several types of ELISA procedures have been developed in the past and several studies are in progress on their use for potency testing of rabies vaccines. They have an advantage that they are rapid, robust, precise, inexpensive, highly reproducible and quantitative .
- (iii) Antibody binding test: In this test serial dilutions of antigen are mixed with a constant dose of specific antiserum and the amount of unbound antibody is determined by titration against live virus by using RFFIT.

(These tests have their own advantages and disadvantages)

Safety Testing: Deletion/Replacement

- ▶ Abnormal Toxicity Test: Ph. Eur., FDA, WHO, IPC has recommended the deletion in line with International harmonization.
- ▶ Inactivation Test (Residual live virus testing): Recommendation by certain regulatory authorities like Ph.Eur. to perform the test at bulk stage and to be deleted at final lot stage and that too by using cell culture method.
- ▶ Pyrogenicity Testing: Efforts to be made for its replacement with *in vitro* test like BET/MAT (I.P.- BET)
- ▶ Tests on Virus seed lots: *In vitro* testing used in case of Vet Rabies Vaccines but *in vivo* testing for human vaccines. Assessment for replacement to be taken up for discussion (WHO –already recommended)

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

- ▶ Serum Institute of India Pvt. Ltd. (SIPL)-PUNE is India's largest manufacturer of vaccines and other biotech products.
- ▶ SIPL produces several bacterial, viral and recombinant vaccines. SI also manufactures combination and multivalent vaccines.
- ▶ SIPL 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.

Consistency approach in Rabies vaccine: SIIPL Experience

- ▶ Alternative methods were used for characterization of vaccine along with in vivo methods.
- ▶ Suitable correlations were developed and were monitored for number of batches.
- ▶ More emphasis on data monitoring of critical parameters, trend analysis.
- ▶ Led to successful implementation of non animal methods without compromising the product quality.

Replacement of In-Vivo tests by In-Vitro Tests for Rabies Vaccine and Rabies monoclonal antibody

In process

Rabies Virus quantitation
(Harvest)
Amplification Test
Identity Test



In process

Fluorescent Antibody Test
(FAT)

Final bulk/lot

NIH Potency Test



Final bulk/lot

Single Radial Immunodiffusion
Test (SRID)

For Rabies Monoclonal Antibody

Antibody estimation

Mouse neutralization
test (MNT)



RFFIT

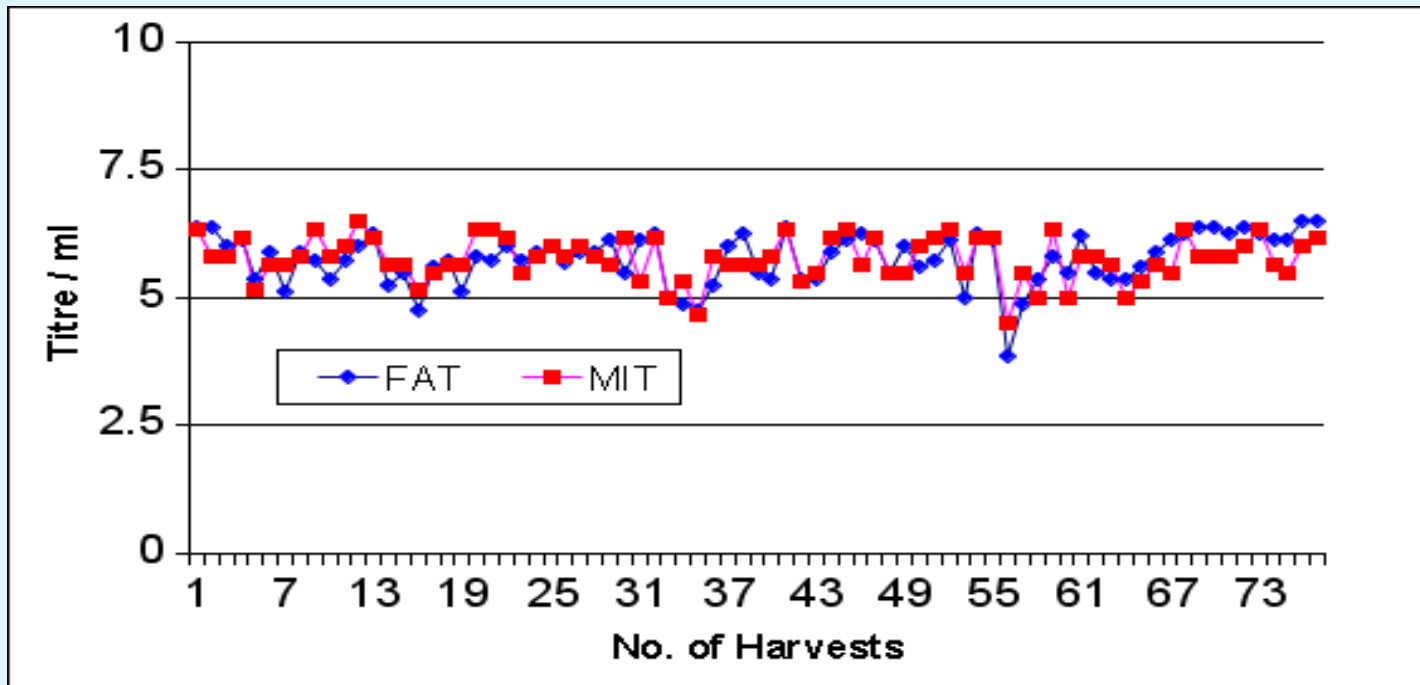
(Rapid Fluorescent Focus
Inhibition Test)

Replacement of In-Vivo tests by In-Vitro Tests in Rabies Vaccine – IPQC

Test	Potency	Animal model	Duration of Test (days)	Number of animals per lot	Annual consumption
Rabies harvest titration	MIT (i.c. challenge method)	Mice	14	18	13824 for 768 harvests
		Mice	14	174	16704 for 96 lots
Amplification test		Mice	14	20	1920 for 96 lots
Identity test (Pooled harvest)					
3 Rs alternative					
As an example	Potency	Model	Duration Test (days)		
Tests on harvests	(FAT)	In vitro using BHK cells	4 days	NA	

Animal reduction by 32448 for 96 lots produced in a year

COMPARATIVE STUDY: FAT and MIT



FAT			MIT		
N	Geometric mean	SD	N	Geometric mean	SD
77	5.7714	0.4940	77	5.7756	0.4178
(Statistics)					
Parametric		Non parametric			
Paired t test		(Wilcoxon's signed rank test)			
0.0951, P=0.9245		1377.0, P=0.9549			

SRID Assay for Rabies Glycoprotein

□ Challenges

Non availability of anti-sera/monoclonal antibody against glycoprotein

□ Progression

✓ In-house development of protocols for isolation of glycoprotein antigens from the virus.

-Tangential flow filtration, sucrose gradient centrifugation and dialysis based techniques to concentrate the virus.

-Isolation and purification of glycoprotein based on detergent treatment (Mulgofen)

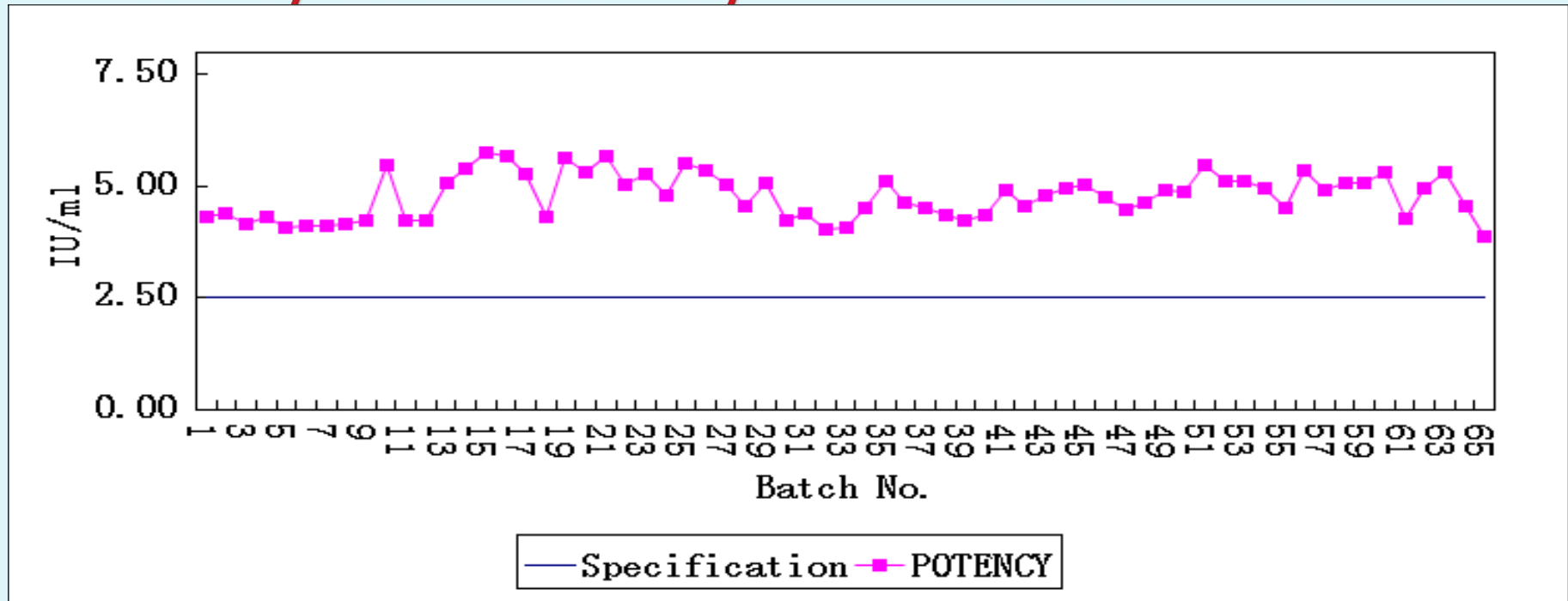
✓ Development of antiglycoprotein sera in sheep.

- Calibration of sera against international standards

✓ **Successful implementation of SRID assay in final bulk resulted in annual saving of 3360 animals annum. (mice).**

Outcome of Consistency approach

Potency estimated by NIH method: Final lot



Trends of potencies estimated by NIH method of 65 final lots which were monitored by critical parameters. This data is representative of lots manufactured in SIIL over a period of 2 years.

Perspective

SILL 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, pharmacopoeias
- Harmonization of regulatory requirements: Important for global supplier like us.
 - **Looking forward to participate in BSP 148**

Reference:

Three Rs Approaches in the Quality Control of Inactivated Rabies Vaccines.

The report and recommendations of ECVAM Workshop 48.

ATLA 31,429–454,2003

Thank You



Consistency approach for routine lot release of vaccines

- Aims to identify critical indicators of safety and efficacy that can replace the need for extensive testing during release.
- Makes use of quality systems to identify critical parameters indicating product consistency.
- Encourages the application of newer concepts such as quality by design and PAT approaches for quality assurance of vaccines.
- SII successfully implemented such approach for rabies vaccine production control.
- Rabies vaccine case study represents an interesting case study wherein combined outcome of 3Rs and consistency approaches can be evaluated.

