Animal testing replacement for vaccines
A One Health View: Global outlook and future strategy

Implementation of non-animal testing and efforts to promote global alignment - An Industry Perspective

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Scope

This presentation provides a brief overview relating to implementation of 3Rs i.e. phasing out of animal assays and phasing in of Non Animal Test Methods (NAMs) in vaccine testing at Serum Institute of India.

- It identifies the tests of most concern from an animal welfare point for view, where refinement of methods, reduction and replacement of animals should be a priority.
- It shows how the positive intention of implementation of 3Rs in collaboration with the Regulatory Authorities (NRA /NCL / Pharmacopoeia commission) could serve as a powerful tool in reducing animal use and suffering.
- Regulatory guidelines - if they are written in a less ambiguous and more consistent way certainly aids in reducing the numbers of animals used in quality control testing by avoiding unnecessary testing.

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

- SIIPL is committed to the development, introduction, validation, and implementation of 3Rs (Refinement, Reduction, and Replacement) and consistency based approaches.
- NRA/NCL and India Pharmacopoeia has always been supportive and receptive to such initiatives.
- SIIPL has been a frontrunner in implementation of 3Rs since 2003 even when Regulatory guidelines did not recommend any alternative.

3Rs Opportunities and implementation at SIIPL

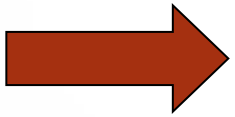
Let us have a look at some of the initiatives taken and implemented

DTP GROUP OF VACCINE (Single Dilution/Serological assays): Replacement

CONVENTIONAL METHOD

Lethal Challenge test

Animals used : Guinea pigs /Mice



ASSAYS FOR 3Rs APPROACH

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

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

Steps in process of test development	Timelines (Diphtheria)	Timelines (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 by challenge Assay.** **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

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%

3Rs approaches for Pertussis

- Currently testing of pertussis vaccines largely based on challenge based procedures.

- SIIPL is working on various alternatives:

  -PSPT

  -Histamine challenge sensitization method's deletion from Indian Pharmacopoeia for aP component.

3Rs Opportunity and Recombinant HEPATITIS B Vaccine

- Comparability studies data of *in vitro* and *in-vivo* assay was submitted to National Control Laboratory for review and replacement of *in vivo* assay.

- Approval for *in-vivo* test on one in five lots by NRA in Year 2006 and obtained Complete Waiver for *in-vivo* tests in 2017

- Annual saving of mice: 100 %

Hepatitis B: Regulatory acceptance

Steps towards implementation	Time lines
Assay development	Year 2003-2004
Submission of proposal to National Control Laboratory	Jan 2005
Acceptance by National Control Laboratory	Feb. 2006 for 1 in 5 lots.
SIIPL submitted data in the Year 2014-15 for consideration for further waiver	Based on consistency, NRA approval on 1in 10 lots was obtained in April 2015, which resulted in further reduction in animal usage and approval for complete waiver in obtained in 2017, thus resulting into 100% saving of the animals.

3Rs Opportunity and Inactivated Polio Vaccine

- Sought complete waiver from performing
  RAT IMMUNOGENICITY TEST

- Now performing D Antigen content estimation by ELISA

3Rs in Polysaccharide Conjugate Vaccines: Hib, Meningococcal, Pneumococcal & Typhoid Conjugate Vaccines

- Tetanus/Diphtheria toxoids at pool stage before conjugation is always tested for Specific Toxicity.
- Consistency was established by testing series of batches of carrier protein and bulk conjugates of Hib and Men A and data was submitted to NRA.
- Approval from NRA and subsequently from WHO was obtained and test was discontinued at conjugate bulk/drug product level.
- 3RS: Test: 21 days, Animal saving: 5 G. Pigs/bulk conjugate lots.
- Based on the above approach, similar approval from NRA and subsequently from WHO was obtained for other Conjugate vaccines involving TT/DT as carrier proteins

3Rs Opportunity in other vaccines

- Sought complete waiver from performing MOUSE IMMUNOGENICITY TEST from NRA awaiting WHO Approval in R21 Malaria Vaccine
- Data under submission to NRA for waiver of in vivo test in case of HPV Vaccine
- Also initiated working on *in vitro assays for D and P components*

Abnormal Toxicity Test (ATT) and Pharmacopoeial recommendations

- SIIPL had earlier implemented deletion of ATT for most of its vaccines in compliance to I.P. addressing it through Post Approval Changes route.
- Finally, in July 2020, the IPC published the Amendment List-0-6 to IP 2018 which stated the removal of ATT from the monographs of all human vaccines.
- However, Global regulatory harmonization is required for such tests. This test is still a requirement for product registration and lot release in different countries.

…..The way forward

However, ATT is still a requirement for product registration in many countries. Therefore, global regulatory harmonization is required for such tests.

- <u>The main factors as we understand which leads to lack of global harmonization are:</u>
- Different NRAs are involved which are guided by their own Legislative and organizational set ups, different regulatory requirements, guidance and country specific decision-making.
- Differences in requirements exist, in part, due to the fact that respective countries have varying expertise in evaluating and requiring implementation of GMP and Quality Systems, and in oversight and surveillance activities that are needed to effectively monitor manufacturing processes and enforce GMP.
- These factors lead to significant burden in terms of costs, time, resources on those manufacturers that produce vaccines for global market.

Pyrogen Test Deletion

- Approval for Implementation of replacement of Pyrogen Test in Rabbits with BET (LAL's Test) obtained from NRA for most of the vaccines
- Vaccines under development are being tested for Pyrogen as well as BET to generate data for comparison and also to justify the omission of Pyrogen Test at the time of licensure.
- In routine BET is being performed.
- Working on introduction of recombinant Factor C (rFC) / recombinant Cascade Reagent (rCR) in testing of endotoxin content.
- Facing challenges with implementation of MAT

Consistency approach in Rabies vaccine

- 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 Q C

Rabies Virus quantitation (Harvest)

Amplification Test

Identity Test

→ In process Q C

Fluorescent Antibody Test

(FAT)

Final bulk/lot

NIH Potency Test

→ Final bulk/final Lot

ELISA

Antibody estimation

Mouse neutralization test (MNT)

For Rabies Monoclonal A'body

→ RFFIT

Replacement of In-Vivo tests by In-Vitro Tests in Rabies Vaccine (Data of one particular year)

Test	Potency	Animal model	Duration of Test (days)	Number of animals per lot	Annual consumption
Rabies harvest Titration (virus concentration)	MIT (I/C challenge method)	Mice	14	18	13824 for 768 harvests
Amplification Test (Inact.Test)		Mice	14	174	16704 for 96 lots
Identity test (Pooled harvest)		Mice	14	20	1920 for 96 lots
Implementing 3 Rs alternative					
	Potency	Model	Duration Test (days)		
Tests on harvests	(FAT)	In vitro using BHK cells	4 days	NIL	

Animal reduction by 32448 for 96 lots produced in a year

Collaboration with Regulators

Efforts to promote Global Alignment

- The industry is actively engaged in collaborative efforts to drive global alignment and acceptance of Non Animal Testing. Key Efforts include:
- Collaboration with Regulators: Industry is working with regulatory bodies through public private partnerships and multistakeholder roundtables to develop roadmap, share data and build confidence in NAMs.
- In India- Collaboration with Regulatory Authorities (NRA/ NCL/ Pharmacopoeial Commission) at a common joint forum served as a powerful tool in reducing animal use and suffering and paved way for implementation of 3Rs in the country.

Collaborative efforts to drive global alignment

Indian Pharmacopoeia commission had been proactive in :

(1) Constituting Experts Working Group on 3Rs **(2)** Publishing the chapters on substitution of animal assays with in vitro assays i.e. General chapter on alternative methods, Replacement of RPT with in vitro assays etc. and now **(3)** In the process of publishing a chapter on NGS etc.

- NRA/NCL extend support in terms of openness and willingness in implementation of 3Rs and expedites the process with faster review and imparting suggestions where ever required and grants approval.

- **International Initiatives**: SIIPL participated in various collaborative studies like PSPT by DCVMN, BSP Studies by EDQM, different studies from NIBSC, collaborated with NC3Rs to identify the animal tests which could be replaced and submitted the report to WHO, subsequently WHO has published the guidelines on 21st Nov 2025 so as to harmonize testing guidelines and promote the mutual acceptance of data generated by validated non-animal methods.

Challenges & Barriers

Challenges and Barriers: The main challenge is a lack of clear, harmonized regulatory guidance and acceptance across different jurisdictions and sectors. Companies are hesitant to invest heavily in developing and validating NAMs without confidence that the data will be accepted by all regulatory authorities. Some of them are as following:

Technical:

- Challenging characterization of legacy products components e.g. DTP Vaccine
- Lack of experience on product specific validation to be performed by manufacturers and method to be transferred to National Control Laboratories (NCLs) releasing the product

Challenges & Barriers

Technical:

- Different GMP, quality and regulatory compliance maturity of manufacturers, NCLs and National Regulatory Agencies (NRAs)
- Reagents availability and clarity on role of international reference standards in validation studies (no 1:1 correlation)

Regulatory:

- Lack of knowledge, experience and confidence on new assays and data analysis.
- Slow pace in changing local regulations/pharmacopoeias, different standards and lack of alignment
- Strong risk aversion mindset and other non technical biases of policy makers and NRAs

Others:

The significant initial investment required for new technologies and training.

Gearing up to speed up the process of phasing in of Non Animal Test Methods (NAMs)

Need to Prioritize Methods Development & Validation

- Leverage successful case studies, knowledge, experience and training
- New projects where manufacturers and NCLs collaborate together to develop, assess, validate a new method.
- Facilitate method transfer for already validated methods

Business & investment Planning

- Support Investment on trainings for implementation of 3Rs
- Facilitate local technology hubs and global reagents suppliers to facilitate access to critical reagents at affordable prices.
- Improve reagents and new technologies availability and affordability.

Regulatory Readiness:

- Promote early dialogue between manufacturers and regulators on non animal testing strategies for novel products and legacy ones (PACs).
- Increased number of international agencies, regulatory agencies, pharmacopoeias and legislation are now including alternative methods or are in the process to include them.

GOAL

- Ultimately, the goal is to create a harmonized global framework where non-animal methods are the default approach for safety assessment, with animal testing used only as a true "last resort".

Thank You!