Manufacturers approaches to implementations of updated DTwP testing strategies

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Transitioning DTwP containing vaccines to animal free batch release testing strategy. Strategies for implementation.

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Scope

- This presentation provides a brief overview relating to implementation of updated testing strategies for DTwP vaccines testing at manufacturer, country, regional and global levels
- It identifies the tests of most concern from an animal welfare and animal use point of view, where Refinement of methods, Reduction and Replacement of animals should be a priority.
- It shows how the positive intention of implementation of those approaches in collaboration with the Regulatory Authorities (NRA / NCL / Pharmacopoeia commission) could serve as a powerful tool in reducing animal use and suffering.

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

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

Consistency Approach

- 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.

3Rs successfully implemented in

Lot Release testing of

- **DT based combination vaccines**
- Rabies Vaccine
- Hepatitis Vaccine
- Polysaccharide Conjugate Vaccines
- Inactivated Polio Vaccine

Deletion from almost all the vaccines:

- Rabbit Pyrogen Test (replaced with BET)
- Abnormal Toxicity Test

An overview of ongoing changes in India

Manufacturers have active 3Rs programs

- Many have implemented more than one 3Rs opportunity for the batch release (safety and potency) like:
  - Single dilution assay for D and T
  - Replacement of Rabbit Pyrogenicity Test (RPT) with Bacterial Endotoxin Test (BET)
  - In vitro potency for Hepatitis A and B
  - Deletion of the Abnormal Toxicity Test
- Manufacturers are committed to invest in improve their 3Rs Program, including the transition to in vitro potency test for DT (aP) and to find a replacement strategy for wP.

An overview of ongoing changes in India

- Collaboration with the Regulatory Authorities (NRA/NCL/Pharmacopoeia commission) at a common joint forum served as a powerful tool in reducing animal use and suffering and pave way for implementation of 3Rs in the country.
- Indian Pharmacopoeia commission had been proactive in:

(1) Constituting Experts Working Group on 3Rs

(2) Publishing 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.

- 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.

Current Situation of 3Rs Industry Implementation in DCVMN Network

- Increased number of manufacturers with active 3Rs programs and with 3Rs methods implemented (e.g. single dose/single dilution for DT based combination vaccines; deletion of ATT; in vitro potency tests for Hepatitis A and B and for Rabies; D-antigen for IPV, replacement of RPT)
- But there are different approaches in prioritizing replacement opportunities vs other 3Rs.
- Need to align locally, regionally and globally on further investment for method development and implementation (DTaP, DTwP) and on sharing expertise and experience on available methods for other animal tests as well

Opportunities to be implemented and explored

- **Single dose / Single dilution for DT based vaccines**
- Replacement / deletion of Specific Toxicity (D/T/P)
- **In vitro potency for DT**
- **aP and wP in vitro potency (wP refinement (short term) vs replacement (long term) strategies)**
- **Combined in vitro potency assay for DTaP or DTwP**
- Rabies in vitro potency
- rFC
- MAT
- NGS (adventitious viruses and for MNVT)
- ATT deletion for non-indigenous market

Application of alternative Methods - D & T Potency Assays (a case study)

Assay	Old Method for potency assay	Alternative method implemented
Potency assay of Diphtheria and Tetanus component in combination vaccines	WHO Lethal challenge assay in Guinea Pig	Single dilution / Single Dose serological T-ELISA (For Tetanus component) and VERO cell assay (For Diphtheria component

COMPARISON - POTENCY ASSAYS

WHO Challenge Assay

- Immunisation (3 goups of animals each) along with reference standard
- After 4 weeks, all animals are challenged with 100 LD50 dose of toxin
- Animals are observed for 5 days further after lethal challenge
- Survival / Total no. of animals ratio is considered for potency estimation
- Relative potency is calculated using Combistats

Serological Assay (Single dilution / Single Dose)

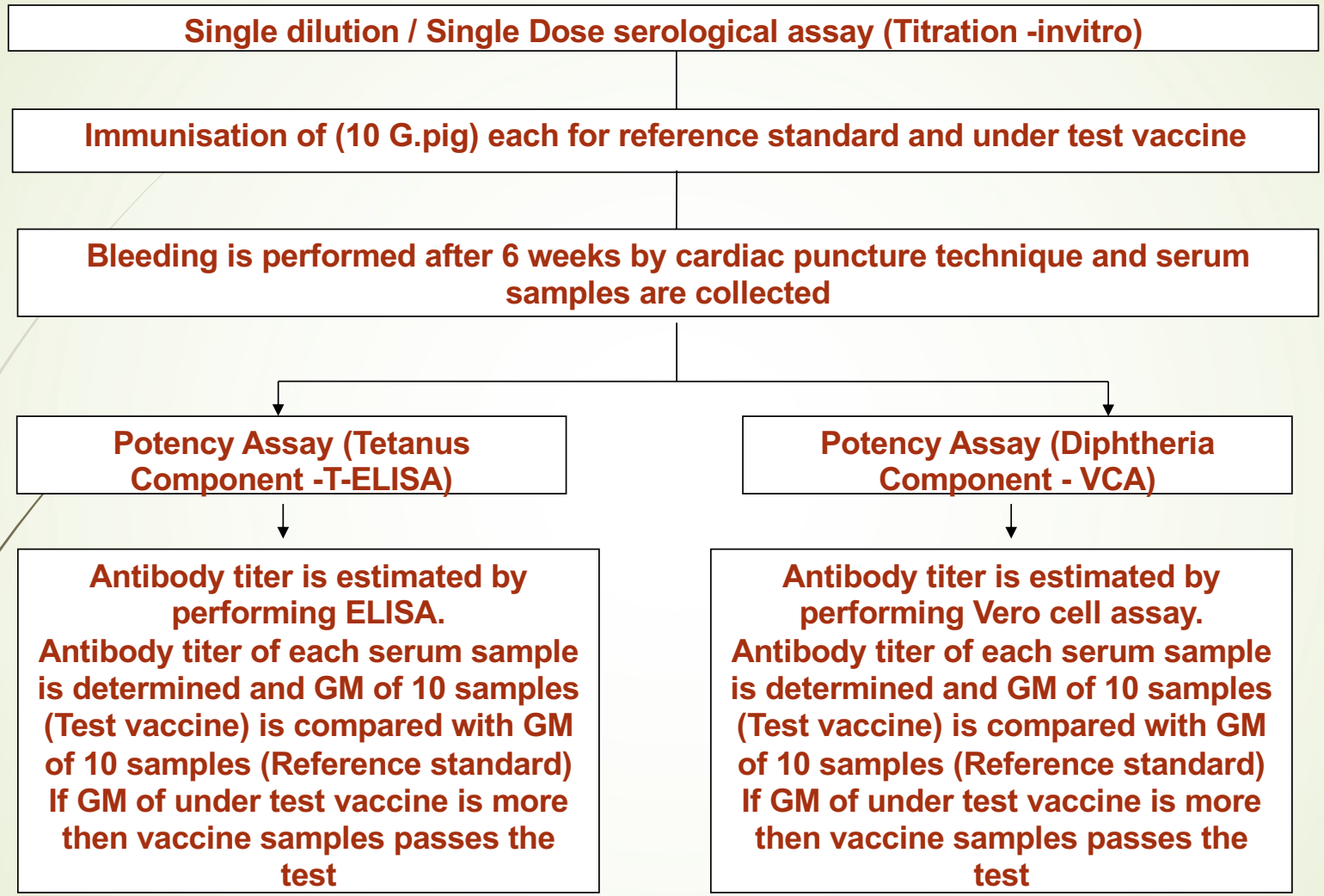
- Immunization of animals with specific dilution of Ref. Standard and vaccine
- Bleeding is performed by cardiac puncture and serum samples are collected

Serological assay's

For Diphtheria: Two fold dilutions of sera samples are titrated against diphtheria toxin (Eg. Ltc/100 dose) and presence of residual un-neutralized toxin is detected using Vero cells.

For Tetanus: Antibody concentration in sera samples is determined by ELISA

ALTERNATIVE POTENCY ASSAY - FLOW



Presentation of case study

Which 'R' is implemented and on which product ?

Which 'R'	Method – WHO Lethal challenge in guinea pig	Alternative method implemented – Serological assay
Reduction in number of animals	112 guinea pigs (Each for Diphtheria and Tetanus component) required to test one batch of vaccine Total – 224 number of guinea pigs/batch	Total 30 guinea pigs/batch are required for the testing of both D and T components.
Approx. 85 % reduction in animal consumption		
Product	DT based combination vaccines	DT based combination vaccines

Benefits of change / implementation of alternative methods

Parameter	Method – WHO Lethal challenge in guinea pig	Alternative method implemented – Serological assay
Consumption of less animals / Cost effectiveness ----------	112 guinea pigs (Each for Diphtheria and Tetanus component) required to test one lot of vaccine Total – 224 number of guinea pigs/lot	Total 30 guinea pigs are required to test each lot of vaccine Approx. 85 % reduction in animal consumption Elimination of distress/trauma in animals
Availability of space animal house / Possibility to test more batches in the existing capacity of animal house		

wP Potency, DT in vitro Potency

- Pertussis Serological Potency Test - the DCVMN project (report in publication) showed that the protocol might work if further improvement to the methodology is carried out and if the regional or international reference is not used for the product specific validation
- wP in vitro potency – method not available but possibility to invest in the development of the methodology.
- Discussion on the best strategy to be considered: short-term with PSPT and long-term with possible new in vitro method?
- DTaP in vitro potency (from the VAC2VAC project) - some DCVMN manufacturers are in the process to test the methodologies developed within VAC2VAC

Open Challenges to phase out animal testing in DT based Vaccines

Technical:

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

Regulatory:

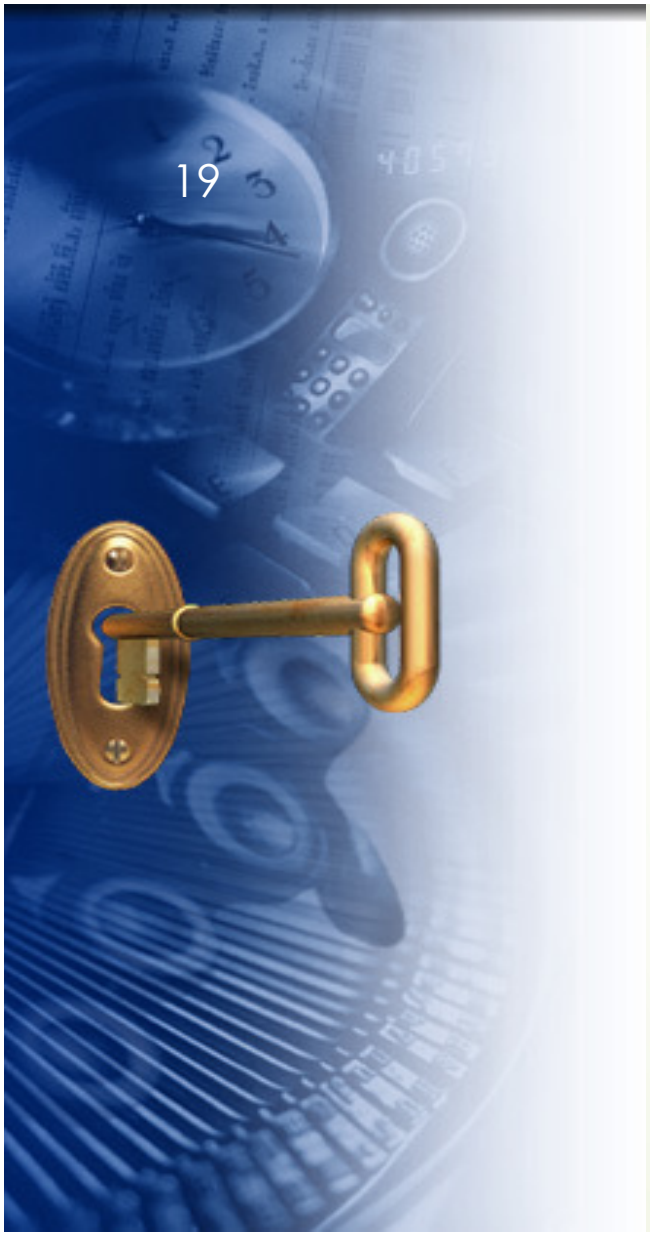
- Lack of knowledge, experience and confidence on new assays and data analysis.
- Slow pace in changing local regulations/pharmacopoeias, different standards and lack of alignment &
- Strong risk aversion mindset and other non technical biases of policy makers and NRAs

Journey for the change has started

- Leverage the international projects and studies (e.g., VAC2VAC, DCVMN PSPT, EDQM BSPs, PATH, NIIMBL etc.) demonstrated the concrete opportunities to use methodologies to replace, reduce and refine animal testing and other international projects to drive implementation (e.g., HSI/AFSA, and NC3Rs) via multi stakeholders dialogue and engagement
- Updated guidelines and registrations in many countries, including the developing countries

Identified Needs to Carry Out the Change

- Investment on trainings
- Sharing SOPs or facilitate method transfer
- Improve reagents and new technologies availability and affordability
- Complexities related to vaccine manufacturers' structure (private companies vs public institutes), local legislations, and the way regulators and pharmacopoeias operate
- Promote early dialogue between manufacturers and regulators on non animal testing strategies for novel products and legacy ones
- Agreement on data driven planning (animal reduction targets, investment on innovation) and on data sharing with regulators
- Need to kick-start new local or international projects or new business opportunities



Thank you