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Application of the 3Rs concept to replace, reduce and refine the use of animals in Quality Control of Vaccines

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Deletion of Abnormal Toxicity Test (ATT)
(Innocuity test)

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Abnormal toxicity test procedure:
Five healthy mice weighing 17-22 grams and 2 healthy Guinea pigs weighing 250-350 grams is injected intraperitoneally and observed for 7 days for any abnormal symptoms or death.

Product/Antigen	Test	Previous practice	Current practice based on NRA approval	Animal reduction /rescue
Tetanus vaccine (Adsorbed)	Abnormal Toxicity Test (ATT) (Innocuity test)	Every batch (5 MICE +2 G. pigs)	Test deleted for routine lot release. NRA approval based on manufacturing consistency. **Note** : ATT test shall be considered during safety related market complaint and critical manufacturing changes. As per Indian Pharmacopoeia, the ATT should comply the requirements when tested by NRA.	100% animals rescued (3R category : Reduction)
Diphtheria, Tetanus, Pertussis (DTwP) group of vaccines				
Hepatitis B Vaccine (rDNA) (Adsorbed)				
Rabies Vaccine (Human)				

Recombinant Hepatitis B vaccine: *in-vivo* potency test (-waiver for reduced testing)

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***In vivo* potency Testing Method :**

Groups of 20 mice (Balb/C strain), weight range 17-22g are immunized intraperitoneally with a series of 4 dilutions of reference and test vaccine using placebo as diluent. After 28 to 32 days individual mice sera are collected and assayed for antibodies to HBsAg using validated ELISA. The relative potency is calculated statistically using Combistat software.

As per IP, BP and WHO, alternative to animal potency test, a validated *in-vitro* method can be used to determine the vaccine potency.

Product/Antigen	Test	Previous practice	Current practice based on NRA approval	Animal reduction/rescue
Recombinant Hepatitis B vaccine	*In-vivo* potency	Every batch 180 Mice/batch	Every fifth batch NRA approval based on data submitted for • Batch consistency • *In-vitro Vs In-vivo* correlation • Method validation data	80% animals rescued (3R category: Reduction & Replacement)

Deletion of Specific Toxicity Test (SPT) *at Final Vaccine stage*

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Specific toxicity test procedure:

Pertussis : 10 mice injected intraperitoneally with ½ SHD + 10 mice injected with 0.5ml saline and observed for 7 days for MWG.

"D" &"T" : 5 Guinea pigs injected subcutaneously with a quantity equivalent to at least five single human doses. and observed for any symptoms of specific intoxication

Observation period : 6 weeks incase of combined "D" &"T" and 21 days incase of "T".

Product/Antigen	Test	Previous practice	Current practice based on NRA approval	Animal reduction/rescue
Tetanus vaccine (Adsorbed) Diphtheria, Tetanus, Pertussis (DTwP) group vaccines	Specific toxicity test for combined "D"&"T" and "P"	Test performed at final vaccine stage and individual antigen stage (10+10 MICE ("P") 5 G. pigs for "D" &"T").	Test removed at final vaccine stage *in-lieu* of successful test performed at antigen stage NRA approval based on manufacturing consistency.	100% animals rescued (3R category : Reduction)

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..'am ...rescued...

Thank You