IMPORTANCE OF 3RS PROGRESS IN ACCEPTANCE OF ALTERNATIVE ASSAYS FOR VACCINES

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BRIEF OUTLINE

1. **Principles of 3R's & Why**
2. **Draft Sch M for biologicals**
3. **3R's initiatives for vaccines**
4. **Comparison between Ph.Eur. & IP for vaccines**
5. **Govt of India initiatives for Cosmetics and New Drugs & clinical Trials**

Principles of 3R's & Why

- **Replacement** – Implementing methods which avoid or replace use of animals
- **Reduction** – Changing test design to minimize the number of animals per experiment
- **Refinement** – Methods to minimize suffering & improve animal welfare

Principles of 3R's & Why

- Animal Studies :
  - Non clinical testing
  - Vaccine lot release/ QC vaccines

- Why 3R's :
  - Social concerns
  - High variability
  - Expensive
  - Availability of in-vitro technologies

Draft Sch M-Part IV: GMP requirements of biological products (GSR 999E)

- Biological processes and materials, such as cultivation of cells or extraction from living organisms – may result in variability
- Biological pro cesses may display inherent variability & the range & nature of by product may also be variable.
- QRM principles shall be used to develop the control strategy across all stages of manufacture to minimize variability and reduce the opportunity for contamination and cross contamination.
- Validation of specific and critical manufacturing steps such as inactivation or steps such as virus removal shall be carried out.
- Control usually involves biological analytical techniques which typically have a greater variability than physicochemical determinations.

Draft Sch M-Part IV: GMP requirements of biological products (GSR 999E)

- The combination of variability in starting materials and potential for subtle changes during manufacturing process requires an emphasis on production consistency.
- Robust process & in-process controls is crucial in the manufacturing of biological active substances & medicinal products
- Biological products like any pharmaceutical product shall be manufactured in accordance with requirements of Pharmaceutical quality system based on a life cycle approach, to facilitate innovation & continual improvement. This approach strengthens the link between PD & Manufacturing activities

Quality by design approach

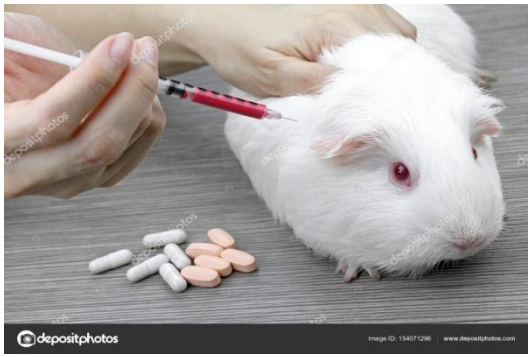
- On-line, in-line, at-line approach as control strategy for consistent manufacturing of biologicals.
- Reduced animal testing, reducing variability in test results.

Recombinant Hepatitis B Vaccine IP 2018

Method B (In Vitro) -

- The potency of the vaccine is determined by ELISA method that has been validated against the biological test. Commercially available kits for measuring HbsAg in-vitro may be used provided they are validated to produce equally precise and accurate results.
- The acceptable criteria are approved by NRA in the light of validation data

Method A(Biological) -



- Intraperitoneal injection in suitable strain of mice /G.Pigs- anesthetize and bleed the animals 28 to 42 later .Assay the individual sera for specific HbsAg antibody concentration by a suitable immunochemical method such as ELISA /RIA

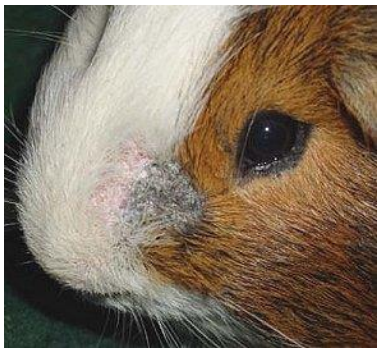
Recombinant Hepatitis B Vaccine

- Based on consistency, CDSCO/NCL approval for 1 in 10 lots and approval for complete waiver subsequently from conducting Method A(Biological) assay for potency

Diphtheria Vaccine (adsorbed)IP

Method A

- Intradermal Challenge test
- The toxin dilution that contains 4x10-5 Lf gives a positive erythema in at least 80% of G.Pigs and dilution that contains 2x10-5Lf gives no reaction in at least 80% of the G.Pigs



Method B - Lethal Challenge method

Method C

- Antibody induction method
- 1/50th of human dose in 10 healthy G.Pigs
- After 2to 3 weeks of 2nd injection ,serum collected to determine diphtheria antitoxin content

Diphtheria Vaccine (adsorbed)IP

- **Method D**
- Any other validated serological assay in G.pigs or mice. Method D, any other validated serological assay in G.pigs or mice as approved by NRA.
- Method A or Method B are used during development of a vaccine ,to assay batches produced to validate the production and wherever revalidation is needed following significant change in the manufacturing process.
- Method A (ID challenge method) or Method B (Lethal Challenge method) may also be used for the routine assay of the batches of vaccine
- Method C (Antibody induction method) can be used where ever possible except during development of a vaccine or revalidation

POTENCY OF DIPHTHERIA COMPONENT - VERO CELL ASSAY - CDSCO/NCL APPROVAL SUBJECT TO FOLLOWING CONDITIONS:

INITIAL Approval :

1. 1 in 10 LOTS needs to be tested by challenge assay
2. Based on good manufacturing practices followed by the firm and knowledge on product performance
3. 1 in 25 LOTS to be tested or once in six months which ever is earlier

Diphtheria Vaccine (adsorbed) IP

- **Bulk purified toxoid**

- Absence of toxin and irreversibility of toxoid
- 1) challenge test in G.Pigs ,the bulk purified toxoid shall pass the test for the absence of toxin, if no G.pig shows symptoms of specific intoxication(diphtheria intoxication-red adrenals) within six weeks of injection and if atleast 80% of the animals survive the test period.
- 2) Alternatively, a cell culture test system may be used, the sensitivity of the test shall have been demonstrated to be not less than that of the G.pig test, and the test procedure shall cell culture method-Vero cells

Adsorbed Pertussis Vaccine (Acellular component): -

- **Test for Absence of residual pertussis toxin and irreversibility of pertussis toxoid:**
- Use not less than 5 Histamine sensitive mice ,Inject 1 human dose by IV or twice human dose intraperitoneally in phos.buffered saline and 0.2 % w/v gelatin ,inject diluent in control mice ,after 5days ,inject histamine base intraperitoneally 0.5ml and observe for 24hrs,if no animal dies ,the preparation complies with the test. Histamine sensitivity of the strain of mice used is verified at suitable intervals: Inject three fold dilutions of a reference pertussis toxin preparation and challenge with histamine.....
- A validated test based on clustering of CHO cells may be used instead of the test on mice

Adsorbed Pertussis Vaccine (Acellular component):-

- **Assay :**
- **CURRENT TESTING PROCEDURE:** Challenge based Procedure
- The capacity of the vaccine to induce formation of specific antibodies is compared with that of reference.
- Healthy mice (eg: CD1 strain),6 groups, ,use 3 dilutions of test and 3 dilutions of reference and attribute each dilution to a group of mice, inject Intraperitoneal or SC,BLEED THE MICE after 4 to 5 Wks of vaccination
- Antibody determination : Assay the individual sera for specific antibodies to each component using validated method such as ELISA
- The test on mice uses a three –point model(3 dilutions of the vaccine) .After validation for routine testing a single dilution method may be used.

Pertussis Vaccine :-

- Alternative tests under development .....
- Histamine challenge based procedures
- Flow Cytometry based methods ....effects on leukocytes

Abnormal toxicity(2.2.1)

- Unless otherwise stated ,all vaccines comply with the test for abnormal toxicity, Method B.(Inject intra peritonially one HD not more than 1ml in each of five healthy mice and one HD ,not more than 5ml into each of two healthy G.pigs.The prep. Passes if none of the animals dies or shows signs of ill health in 7 days, repeat...) In vaccines containing Phenol as preservative ,the test in mice may be inappropriate
- Abnormal Toxicity test may be omitted for routine release once the consistency in production has been well established/demonstrated to the NRA and when Good Manufacturing Practices are in place. Each lot, if tested by the NCL should pass the test for abnormal toxicity

Principles of 3R's for Vaccines - Comparison between EP & IP 2018

	EP	IP 2018
General safety test	Waived for routine testing(after consistency is demonstrated)	Waived for routine testing(after consistency is demonstrated)
Specific toxicity for Pertusis Vaccine	Cell based test at DS stage & waiver of test at DP Stage	Cell based test at DS stage & waiver of test at DP Stage
Specific toxicity for Diphtheria Vaccine	Cell based method for DS & Waiver of the test at DP stage	Cell based method for DS & Waiver of the test at DP stage
OPV, Live (Neurovirulence test)	Switching from NHP to transgenic mice	Neurovirulence in NHP for master & working seed lot – TgPVR21 transgenic mouse model alternative to monkey neurovirulence

Principles of 3R's for Vaccines - Comparison between EP & IP 2018

	EP	IP 2018
IPV (inactivation test)	Primary monkey kidney replaced with L20B cell line	Test for effective inactivation Residual live poliovirus by inoculation on suitable cell cultures
Diphtheria & tetanus vaccine potency assay	Serological tests replaces lethal end points	Serological tests replaces lethal end points
IPV (Assay)	In vivo assay replaced with ELISA	D-antigen content for human poliovirus 1,2 & 3 immuno-chemical method 2018 IP For vaccines not yet approved results of rat assay on all final bulks should be included in all data generated for demo of consistency before waiving in-vivo assay.

Principles of 3R's for Cosmetics

- India has become the **first country** in South Asia to ban animal testing for the manufacture of cosmetics. Dr. G N Singh, the Drug Controller General of India, announced the decision at a Cosmetics Sectional Committee meeting of the Bureau of Indian Standards (BIS) on June 28. The ban is based on an application by **People for Ethical Treatment of Animals (PETA)** which has been campaigning for the ban on animal testing for a long time. PETA in its application also argued for the relevant alternatives to the tests.
- The ban is effective on wide range of products that include lipsticks, eye make-up, and toothpaste. However, India is yet to ban marketing of animal tested products manufactured in other countries. BIS will now have to set down standards to meet the new requirements while the DCGI will make relevant amendments in acts and laws.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News / India / Taking the lead: India becomes first South Asian country to ban animal testing for cosmetics

Taking the lead: India becomes first South Asian country to ban animal testing for cosmetics

In a decision of rare unanimity, the Bureau of Indian Standards has decided to remove animal testing as a legal and legitimate standard for cosmetics. Going a step further, to avoid exploitation of loopholes, the Bureau has also made alternative non-animal tests mandatory.

Principles of 3R's for Cosmetics

- **Alternative tests and technologies available -** Between August and December 2012, India banned several tests on animals. The current ban pertains to the two remaining tests, namely acute oral toxicity limit test and oral mucosal irritation test. "These tests can easily be replaced by computer simulations and tests on human or animal cells," says the marketing-director of an Indian manufacturer which stopped using tests on animals a few years ago

Principles of 3R's for Cosmetics

- Rule **148-C.PROHIBITION OF TESTING OF COSMETICS ON ANIMALS**
- No person shall use any animal for testing of cosmetics
- W.e.f 21-5-2014

Principles of 3R's for Cosmetics

- Rule 135-B. Prohibition of import of cosmetics tested on animals.-
- No cosmetic that has been tested on animals after the commencement of the Drugs and Cosmetics (Fifth Amendment) Rules, 2014 shall be imported into the country.
- W.E.F 12-11-2014

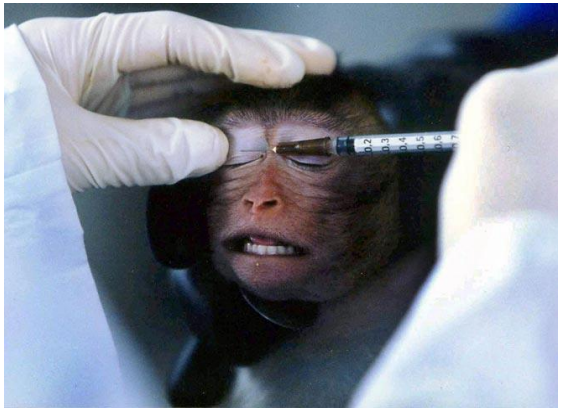
India Ends Re-Testing of Drugs on Animals for New Drug Registrations

- In Schedule Y to the said rules, in Appendix I, in item 4, after sub-item 4.8, the following note shall be inserted, namely: Note.
- Where the data on animal toxicity as per the specifications of Appendix III has been submitted and the same has been considered by the regulatory authority of the country which had earlier approved the drug, the animal toxicity studies shall not be required to be conducted in India except in cases where there are specific concerns recorded in writing.
- Ins.by **GSR 313(E), dt. 16-03-2016**

Rule 75 of New Drugs & clinical Trials 2019

- CHAPTER X IMPORT OR MANUFACTURE OF NEW DRUG FOR SALE OR FOR DISTRIBUTION

- **75(8) -** The submission of requirements relating to animal toxicology, reproduction studies, teratogenic studies, perinatal studies, mutagenicity and carcinogenicity in the application referred to in sub-rule (1), may be modified or relaxed in case of new drugs approved and marketed for more than two years in other countries, if the Central Licensing Authority is satisfied that there is adequate published evidence regarding the safety of the drug, subject to other provisions of these rules.



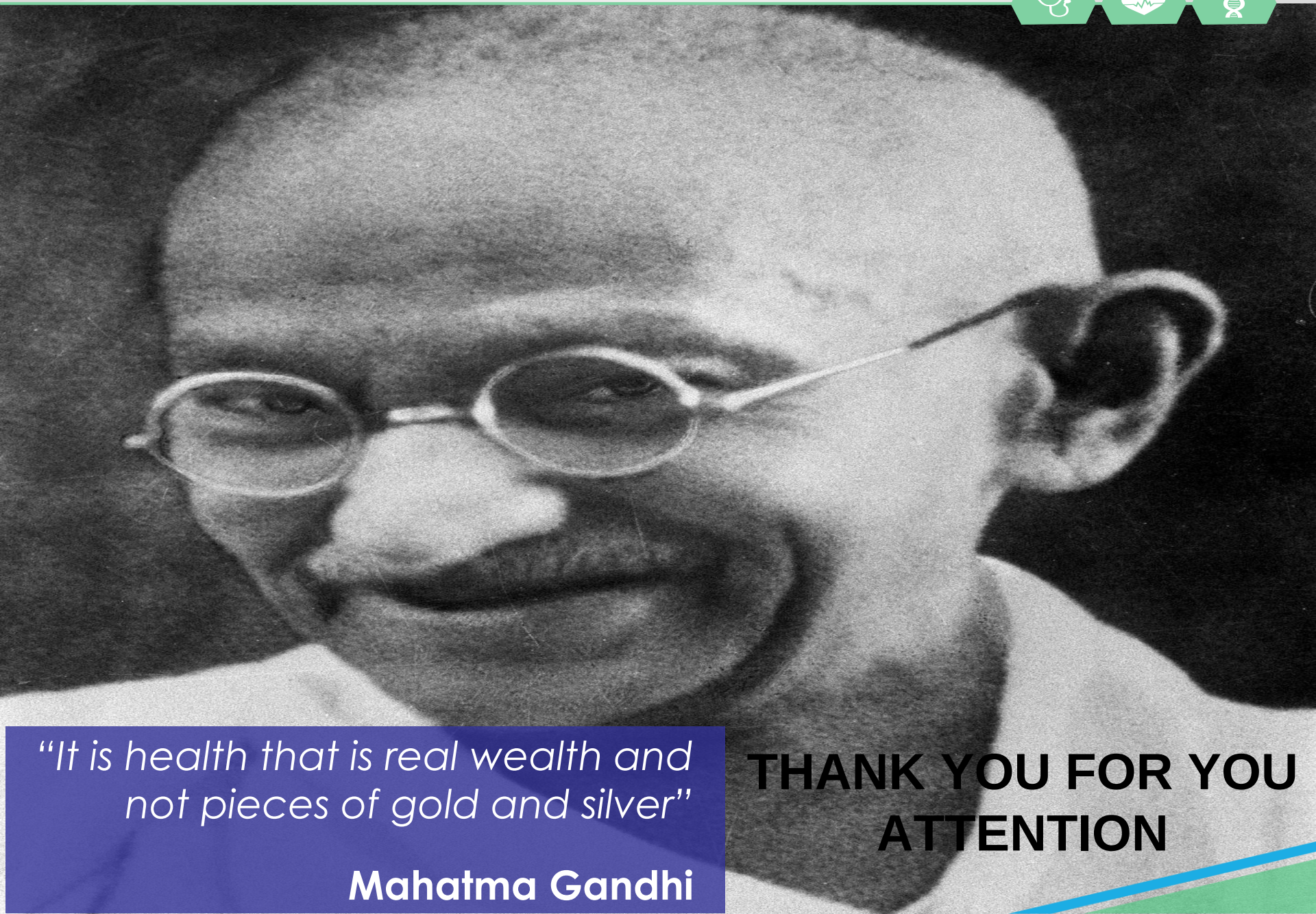
Rule 80 of New Drugs & clinical Trials 2019

- The **Central Licensing Authority** may relax this condition, where the drug is indicated in life threatening or serious diseases or diseases of special relevance to Indian health scenario or for a condition which is unmet need in India such as **XDR tuberculosis, Hepatitis C, H1N1, Dengue, Malaria, HIV,** or for the **rare diseases** for which drugs are not available or available at a high cost or if it is an orphan drug
- In the application referred to in sub-rule (1), the submission of requirements relating to **animal toxicology, reproduction studies, teratogenic studies, perinatal studies, mutagenicity and carcinogenicity** may be **modified or relaxed** in case of new drugs approved and marketed for several years in other countries, if the Central Licensing Authority is satisfied that there is adequate published evidence regarding the safety of the drug, subject to other provisions of these rules.

Rule 80 of New Drugs & clinical Trials 2019

- The animal pharmacological and toxicological data and clinical data needed in such cases will usually be determined on **case-by-case basis** depending on the type of new claims being made by the applicant as well as the mechanism of action, pathophysiology of the disease or condition, **safety and efficacy profile in the respective conditions or population and clinical data already generated with the drug in the approved claim**. requirements may be **abbreviated or relaxed or omitted** as considered appropriate by the Central Licencing Authority under following conditions:
- (a) *the drug is already approved and marketed in other country for the proposed new claim;*
- *(b) clinical data supporting the benefit-risk ratio in favour of the drug in the proposed new claim is available;*
- *(c) the clinical trial doesn't involve a route of administration, dose, patient population that significantly increases the risk associated with the use of the drug*.

Celebrations



"It is health that is real wealth and not pieces of gold and silver"

Mahatma Gandhi

THANK YOU FOR YOU ATTENTION