Modern science for 3Rs alternative approaches and better quality control of human vaccines

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Current animal use for Vaccines

- **Testing of Biologicals** has the highest proportion and number of experiments causing severe pain and distress to animals out of various types of experiments (basic research, toxicity testing, etc.).

- **Animal Use in Vaccine industry**
  - Vaccine development (Research, non-clinical evaluation of safety & efficacy)
  - Production
  - Batch control testing (safety and potency testing)

- **Vaccine batch control testing** is responsible for ~90-95% of animal use by vaccine manufacturers.

- In addition, independent Batch Release testing by National Control Laboratories.
Animal Use at Sanofi Pasteur: Current View

- **Sanofi Pasteur**: the first user of laboratory animals within Sanofi group (~75%)

- 90 to 95% of animals used in Industrial Affairs

- 99.4% are rodents

- 2 fold decrease of number of animals used for marketed vaccine testing since 2003

- Stable since 2008 due to increased quality, regulatory requirements and types of vaccines supplied
Why change?

Animal welfare
- Animals are sentient beings
- Large % of animals used in vaccine QC exposed to severe pain & distress
- Societal concern of animal usage

Science
- *In vivo* models act as a black box
- Relevance to human is sometimes questionable
- Lack of robustness, many factors may affect outcome

Economics
- *In vivo* tests are expensive
- Long cycle times
- High variability can lead to rejection of safe and efficacious vaccines, delays in product release & vaccine shortages

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3Rs - Legal basis in Europe

- EMA/CPMP/SWP/728/95 (adopted 1997): Replacement of animal studies by in vitro models


- Directive 2010/63/EU on the protection of animals used for scientific purposes on 3 June 2010.
  - European Directive 2010/63/EU on the protection of animal used for scientific purpose “Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used …”

- Sanofi policy: “As a key element of Corporate Social Responsibility, Sanofi commits to meet or exceed regulations and standards for the use of animals and to develop alternative approaches. Sanofi fully adheres to the 3Rs…”
A clear 3Rs commitment within Sanofi Pasteur

The objective of Sanofi Pasteur, with the support and commitment of its R&D, is to phase out animal use for the manufacturing and control of marketed products in particular for new vaccine registration and reduce the number of animals used for the development of new products.
## Current Limitations to 3Rs for vaccine quality control

### Regulatory hurdles

- Lack of harmonization of regulatory requirements
- Caution of Health Authorities to accept deviations from established guidelines and monographs
- Risk aversion – *in vivo* assays used for decades. Animal tests considered as “gold standard”
- Complexity of post-approval changes do not generate strong incentive to develop and implement alternatives to animal testing

### Scientific hurdles

- Inherent variability of *in vivo* assays
- Historically *in vivo* assays have not been validated according to current ICH Q2 R1 principles
- The product quality attributes will likely be assessed differently when changing from an *in vivo* to an *in vitro* method
- 1:1 comparison challenging and not necessarily justified
Regulatory hurdles: Many regulatory worlds

One world or many worlds?

USA/Canada

WHO

Brazil

EU

EEU

China

India

Japan

Australia

Local requirements / lack of harmonization
Diphtheria and Tetanus potency assays status

Several methods required/recommended depending on the destination

- **Challenge tests**
  - Guinea pigs for D
  - Mice and guinea pigs for T
    - **Multi-dilution assay**
      - Potency expressed in IU
    - **One-dilution assay (limit test)**
      - estimated potency > minimum requirement
    - **In vivo Toxin Neutralisation Test**
      - US NIH Functional Ab response above threshold

- **Immunogenicity tests**
  - Serology by ELISA or in vitro TNT on Vero cells (for D) or Toxin Inhibition assay (for T)
    - **Guinea Pig model**
      - multi- or one-dilution assay
    - **Mouse model**

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Scientific hurdles

- The test methods used for routine QC should be intended to monitor production consistency and ensure comparability of quality attributes between commercial and clinical lots.
- The information provided by *in vivo* and *in vitro* methods is different.
- An existing *in vivo* method may be substituted by more than 1 *in vitro* method in order to control key qualitative and quantitative attributes measured by the existing test.
- New Ph. Eur. general chapter 5.2.14 “Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines” effective since Jan 2018.

**In Vivo assay**
- Measure Complex functional response

**In Vitro assay**
- Mimic specific elements of complex *in vivo* response

Agreement between methods should not always be expected. Based on the demonstration of the scientific relevance of new test.
Time for change… for consistency approach

1:1 replacement
- Uniqueness of each batch
- Emphasis of QC on final product
- Read out in IU (potency)
- Comparison to international reference preparation

Integrated approach
- Each batch is one of a series
- Emphasis on every step of production process (seed lot, in-process, final product)
- Read out is: non deviation from consistency
- Benchmarking to clinical/ historical batch

‘The **consistency approach** is a concept which includes the strict application of **GMP rules** and guidelines, process **validation** and in process and final product **tests** and is aimed at verifying if a manufacturing process produces final batches which are **consistent** with one that fulfils all the criteria of **Quality, Safety and Efficacy** as defined in the marketing authorization, ultimately resulting in replacement of routinely used in vivo tests. **De Mattia et al, Biologicals 39:59-65, 2011**
Perspectives for the future: in vitro analytical tools

- Standardized Processes
  - Enabling technologies
    - Better knowledge & monitoring
      - Purified antigens
        - Antigenicity
        - Structure
      - Residual toxicity
      - Aggregation
      - Purity
  - GMP
    - Product consistency
  - Final formulated products
    - Antigenicity
    - Content
    - Interaction with adjuvant
    - Residual toxicity
  - Seeds
    - Sequencing
    - Toxinotyping
International collaboration is key for success

- Human Rabies Vaccines (Potency test replacement)
- Harmonisation of 3Rs in Biologicals (deletion GST/ATT)

HIST replacement
2010-2015 international workshops & collaborative studies

NIH
ICVAM
EDQM
NC3Rs

Vac2Vac project
Vaccine batch to vaccine batch comparison by consistency testing

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Integrated 3Rs Strategy for new vaccines

- **Yesterday**, animal models were predominant

- **Today**, we use non-animal data, animal model and clinical research to support the development of new products

- **Tomorrow**, the ultimate objective is to replace the use of animals thanks to translational approaches, breakthrough innovations and Regulatory Science
Conclusion

• Overall reduction in Research and phase out for quality control is a strong commitment

• We still need animals for Research and Development of new vaccines
  • Although progress is being made, this is a long and resource-consuming journey
  • Non-animal methods and animal models are complementary, and the ratio of non-animal ones is increasing
  • Refinement must be maintained when animal are used

• For existing vaccines,
  • a change of mindset is necessary to continue replacing in vivo by in vitro testing: consistency approach
  • Need for a worldwide harmonization and commitment from regulatory bodies
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