



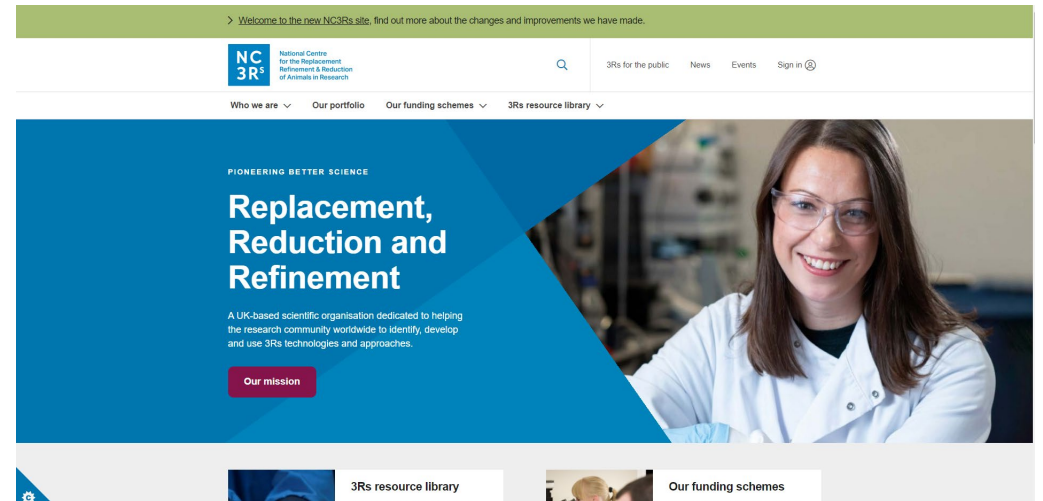
National Centre  
for the Replacement  
Refinement & Reduction  
of Animals in Research

# Review of animal testing requirements in WHO Guidelines and Recommendations for biologics: a proposal to implement 3Rs principles

**Dr Elliot Lilley – Programme Manager**

# The NC3Rs: the headlines

- Established in 2004 to accelerate the development & uptake of the 3Rs
- Research funder plus in-house programmes led by NC3Rs staff
- Work with industry, academia, regulators & funders – not just UK, but also with collaborators in Europe, North America & Asia
- Remit includes any area of animal use for research purposes
- Team based in London, plus regional staff
- Budget ~ £10 million p.a.
- Independent Board



# Aims and objectives of the project

- A scientifically-driven review of animal testing requirements described in WHO guidance documents for biologics and vaccines
- To identify evidence-based opportunities for the integration of the 3Rs
- To support vaccines manufacturers, regulators and control laboratories in applying the latest non-animal testing approaches and 3Rs strategies
- To support faster access to cheaper vaccines
- Co funded by the NC3Rs and BMGF

# Animal use in biologics development and testing

- Animals are used extensively in the quality control and lot release testing of biologicals
- There are significant issues with this, including
  - Large numbers of animals are used
  - Potential to cause considerable pain and suffering
  - Expensive and labour intensive
  - Time consuming and a cause of significant delays
  - A high degree of variability and risk of failure of otherwise acceptable product batches
  - Often poor repeatability between manufacturer and control laboratories
  - Lack of harmonisation in assay requirements

# UK statistics – 2021 Quality control (QC) testing

| Species    | Actual severity | QC: Batch Safety testing | QC: Batch potency testing | Other QC |
|------------|-----------------|--------------------------|---------------------------|----------|
| Total      | All             | 13,406                   | 65,038                    | 15,747   |
| Total      | Moderate        | 485                      | 24,293                    | 275      |
| Total      | Severe          | 73                       | 31,645                    | 496      |
| Mouse      | All             | 12,496                   | 59,586                    | 14,756   |
| Mouse      | Moderate        | 194                      | 23,683                    | 275      |
| Mouse      | Severe          | 73                       | 29,968                    | 496      |
| Guinea Pig | All             | 509                      | 1,911                     | 246      |
| Guinea Pig | Moderate        | 285                      | 370                       | 0        |
| Guinea Pig | Severe          | 0                        | 1,255                     | 0        |

In 2021:

1,726,980 total procedures

94,191 total QC testing

3.4% total procedures were severe

**34.2% of QC testing procedures were severe**

**48.7% of batch potency testing procedures were severe**

## UK statistics -

#### IV.2.2.2.1.1. Quality control related uses

Quality control includes uses of animals in the testing of purity, stability, efficacy, potency and other quality control parameters product (and its constituents) such as vaccines, and any controls carried out during the manufacturing process for registration purposes, to satisfy any other national or international regulatory requirements or to satisfy the in-house policy of the manufacturer.

Quality control related uses represented 960,000 uses in 2019. A large majority of these uses were related to batch potency-testing purposes (78%).

With more than 215,000 severe uses (-15% uses compared to 2018), batch potency testing was the most severe type of procedure, representing more than 24% (1% less compared to 2018) of all severe uses in the Union (Figure 19). Pyrogenicity testing is the least severe with less than 1% of severe uses.

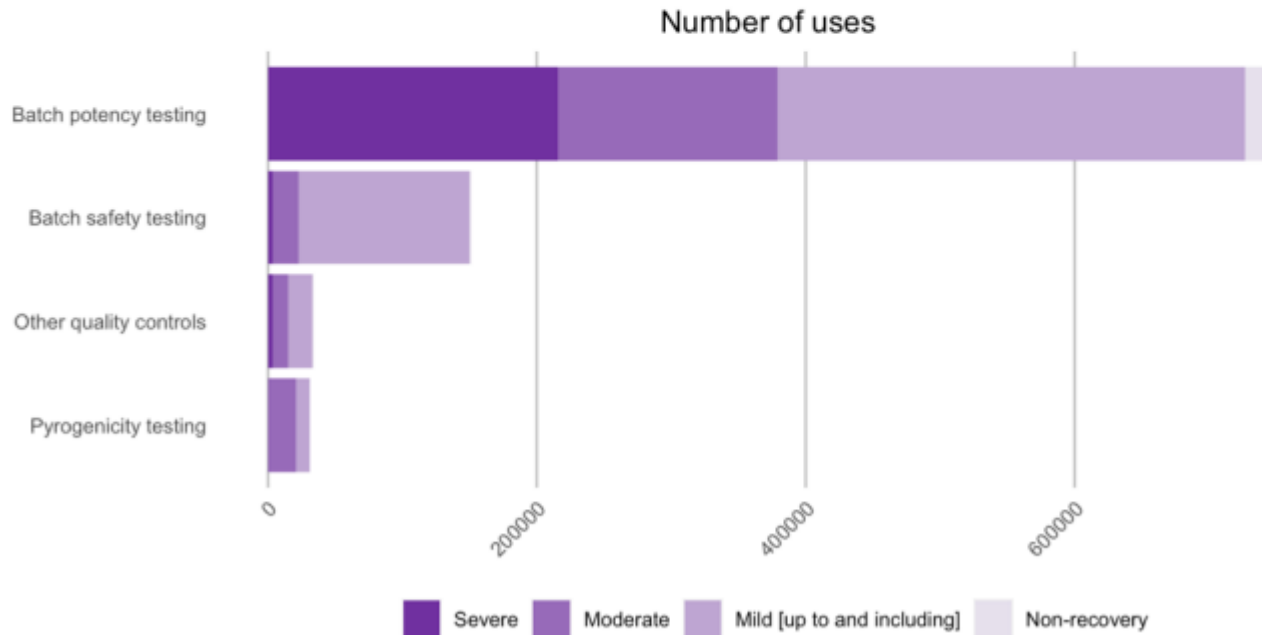
## Procedures Testing

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| Species    | Actual severity |
|------------|-----------------|
| Total      | All             |
| Total      | Moderate        |
| Total      | Severe          |
| Mouse      | All             |
| Mouse      | Moderate        |
| Mouse      | Severe          |
| Guinea Pig | All             |
| Guinea Pig | Moderate        |
| Guinea Pig | Severe          |



**Figure 19: Quality control related uses by type of use and severity in 2019**

# The project

- To review the animal testing requirements described in WHO guidance documents for biologics and vaccines to identify opportunities for the integration of the 3Rs.
  - What is the extent of animal testing included and are there alternative methods that should be included in the recommendations?
  - Would a WHO guideline for the adoption of 3Rs principles into the quality control and lot release of licensed vaccines be useful for harmonisation of non-animal methods and for guidance to WHO member states?
  - What are the barriers that are hindering the adoption of 3Rs principles?



# Formally endorsed by WHO

The project has been endorsed by the WHO Expert Committee on Biological Standardization (ECBS) (World Health Organization. Expert Committee on Biological Standardization, Seventieth report. WHO Technical Report Series. 2020; 1024: Section 2.2.2.).

## 2.2.2 Report from the WHO network of collaborating centres on standardization and regulatory evaluation of vaccines – proposal for implementation of 3Rs principles

Dr Richard Isbrucker presented a proposal to systematically review the animal testing requirements and procedures set out in WHO written standards. Significant issues currently exist in relation to animal testing for in-process, batch-release and stability-testing purposes. Such testing is time consuming, expensive and labour intensive, and leads to significant delays. In addition, it is typically highly variable and increases the risk of failure of otherwise acceptable product batches. Poor repeatability between manufacturer and control laboratories can further delay vaccine batch release. There is also a lack of harmonization in animal-testing requirements across regulatory jurisdictions.

The purpose of the proposed review would be to determine how much and which animal testing should be included in WHO documents for biologicals and vaccines. An assessment would also be made of whether relevant 3Rs strategies are currently available that have not been considered within existing WHO documents. The review process would seek to determine if a WHO strategy for the adoption of 3Rs principles would be useful to NRAs, national control laboratories (NCLs) and manufacturers, and would investigate barriers to the adoption of 3Rs principles.

The review would be conducted in two stages. Stage 1 would be led by the National Centre for the 3Rs (NC3R) in the United Kingdom. This scientific



# Project process

## Stage 1 – NC3Rs

- Review and recommendations
- Formation of an expert working group
- Review of WHO Guidelines
- Recommendations for integration of 3Rs principles
- Identify barriers for adoption
- Stakeholder engagement workshops

**Estimated timeline: 3 years**

Review is submitted to ECBS for their endorsement to proceed to Stage 2

## Stage 2 – WHO

- Drafting & implementation
- WHO working group
- Drafting a response
- Putting the recommendations into practice
- Implementation workshops

**Estimated timeline: 2-3 years**

## Our approach

- Regular stakeholder engagement, throughout the project.
- Change the *emphasis* in WHO guidelines to *promote* adoption of non-animal alternatives.
- Animal tests will only be recommended for deletion with a sound scientific basis.
- General 3Rs guidance will be drafted to promote the scientific benefits of non-animal alternatives, optimised experimental design and high standards of animal welfare.

# Engaging relevant expertise

| National Regulatory Agencies             | Manufacturers          | National Control Laboratories                      | Others   |
|--|------------------------|--|--|
| MHRA                                     | GSK                    | NIBSC, UK  | WHO  |
| FDA                                      | Janssen                | Paul Ehrlich Institute, Germany                    | Seoul National University, S. Korea            |
| South Africa National Control Laboratory | Merck                  | National Institute of Infectious Diseases, Japan   | Eur Commission Joint Research Centre           |
| EDQM, France                             | Sanofi                 | National Institutes for Food & Drug Control, China | IABS   |
| Health Canada                            | Serum Institute India  | Ministry of Public Health, Thailand                | Expert Committee on Biological Standardization |
| ANMAT, Argentina                         | IFPMA, DCVMN           | RIVM, Netherlands                                  |  |
|  | Finlay Institute, Cuba | National Control Laboratory Network                |  |
|  | Biological E           | ANSM   |  |

# Progress to date

## Guideline reviews



| Recommendations to assure the quality, safety and efficacy of BCG vaccines |             |      |             |                   |        |                        |      |      |           |   |               |           |
|--|-------------|------|-------------|-------------------|--------|------------------------|------|------|-----------|---|---------------|-----------|
| WHO guideline title  | TBS         | Year | Product     | Subgroup category | Page # | Section #              | 3Rs? | ADM? | Serology? | Test name                                 | Test category | In Scope? |
| Recommendations to assure the quality, safety and efficacy of BCG vaccines | 979 Annex 3 | 2013 | BCG vaccine | Bacterial         | 142    | General considerations | N    | N    |           | n/a                                       |               | DK        |
| Recommendations to assure the quality, safety and efficacy of BCG vaccines | 979 Annex 3 | 2013 | BCG vaccine | Bacterial         | 148    | A.3.2.2                | N    | N    | N         | Delayed hypersensitivity test             |               | Y         |
| Recommendations to assure the quality, safety and efficacy of BCG vaccines | 979 Annex 3 | 2013 | BCG vaccine | Bacterial         | 148    | A.3.2.5                | N    | N    | N         | Test for absence of virulent mycobacteria |               | Y         |
| Recommendations to assure the quality, safety and efficacy of BCG vaccines | 979 Annex 3 | 2013 | BCG vaccine | Bacterial         | 149    | A.3.2.6                | N    | N    | N         | Test for excessive dermal reactivity      |               | Y         |
| Recommendations to assure the quality, safety and efficacy of BCG vaccines | 979 Annex 3 | 2013 | BCG vaccine | Bacterial         | 150    | A.4.2.3                | N    | N    | N         | Test for absence of virulent mycobacteria |               | Y         |

- 81 guidelines reviewed
- 350 animal tests for batch/lot release testing
- 5 ‘focus groups’ formed to identify 3Rs opportunities

## Stakeholder engagement

- 4 Regional workshops planned in 2022 – Europe\*, Asia\*\*, Pan-America\*\*\*, Africa/Eastern Med. Region
- Survey of biological manufacturers in late 2021 (manuscript submitted to Biologicals)
- Survey of regulatory community in early 2022 (manuscript in preparation)

\* European workshop held on 2 March 2022.

\*\* Asian workshop held on 28 April 2022.

\*\*\* Pan-American workshop to be held on 26 September 2022.

# Focus groups

**Several thematic test categories emerged from the review:**

- Potency/immunogenicity testing
- Pyrogenicity/endotoxin testing
- Neurovirulence testing
- Adventitious agent testing
- Specific toxicity testing

**We have established focus groups to evaluate the potential for adoption of 3Rs principles**

Alternative text emphasising 3Rs for most tests finalised

# Examples

## Pyrogenicity and endotoxin testing

- Well validated non-animal alternatives are available
- Monocyte activation test (MAT) for both endotoxin and non-endotoxin pyrogens – replaces rabbit pyrogen test
- Recombinant factor C test for endotoxin – replaces limulus amoebocyte lysate (LAL) assay.
- Risk-based approach to determine test strategy
- Need to address MAT access/cost issues

## Neurovirulence testing

- In most cases non-animal alternatives are not available (although some are in development)
- Cell-based assay (Mutant analysis by PCR and restriction enzyme cleavage; MAPREC) is available for oral polio vaccine
- Risk-based approach using historical / (pre-) clinical, and pharmacovigilance data, and by data generated with nucleic acid amplification and sequencing techniques to determine genetic stability
- Strategy is to avoid *in vivo* assay when supported by data
- Need to support development/validation of *in vitro* assays

# Stakeholder engagement

## Take-home messages from surveys and workshops

- Non-animal QC tests are, in most cases, quicker, easier, cheaper and more reliable than the current animal-based approaches.
- Many manufacturers are actively developing and using non-animal test methodologies.
- Most regulators are open to receive non-animal test data.
- Lack of international harmonisation is challenging.
- Revised WHO guidelines, with greater 3Rs integration would be welcomed by both regulators and manufacturers
- Similarly, a 3Rs position statement/guideline from WHO (similar to Ph Eur 5.2.14) would also be well received.







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# Thank you!

## For more information

✉ [elliott.lilley@nc3rs.org.uk](mailto:elliott.lilley@nc3rs.org.uk)

🏠 [www.nc3rs.org.uk](http://www.nc3rs.org.uk)

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