Bringing quality control and batch testing of biologicals into the 21st century

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Register for our stakeholder workshop

Introduction

The 3Rs (replacement, reduction and refinement of animals in research and testing) have gained significant traction globally as a framework to support the best, most innovative science. This is especially true right now in the quality control and batch release testing of biological products where large numbers of animals are used globally each year. The assays used in these tests have changed little since they were first introduced but there is now a swell of support for change.

Over the last decade significant strides have been made to apply non-animal approaches in this field and to remove obsolete tests such as the general safety test. However substantial challenges remain to the global adoption of these by some regulatory authorities and manufacturers, which continues to drive the use of animals. Here we describe a project led by the UK NC3Rs to accelerate the transition to 21st century, human relevant testing methods for quality control and batch release testing of biologicals.

NC3Rs WHO project

Biologicals such as vaccines, cytokines, enzymes, and hormones are tested extensively post-licensure as part of routine quality control and batch release testing to ensure the safety and potency of products. The World Health Organization (WHO) is mandated to establish international standards for this purpose and as such their guidelines and recommendations carry significant influence, being adopted by most global regulatory authorities. However, a review of the animal testing requirements within these guidelines has never been conducted and opportunities to adopt the latest non-animal technologies are being missed.

We are working with the WHO, with funding from the Bill & Melinda Gates Foundation, to carry out an independent and comprehensive review of WHO guidelines for biologicals [1] to determine:

- Which animal tests are recommended for the batch release testing of biologicals and vaccines.
- Where 3Rs principles are already encouraged and what opportunities exist for better implementation of the 3Rs and alternative test methods.
- What barriers exist in different regions which may hinder the adoption of 3Rs approaches by manufacturers, national regulatory authorities (NRAs) and control laboratories (NCLs) that are responsible for the testing and release of biologicals.

The NC3Rs will make recommendations to WHO and the Expert Committee on Biological Standardization (ECBS) on how more robust application of 3Rs principles in quality control and batch release testing could be best achieved.

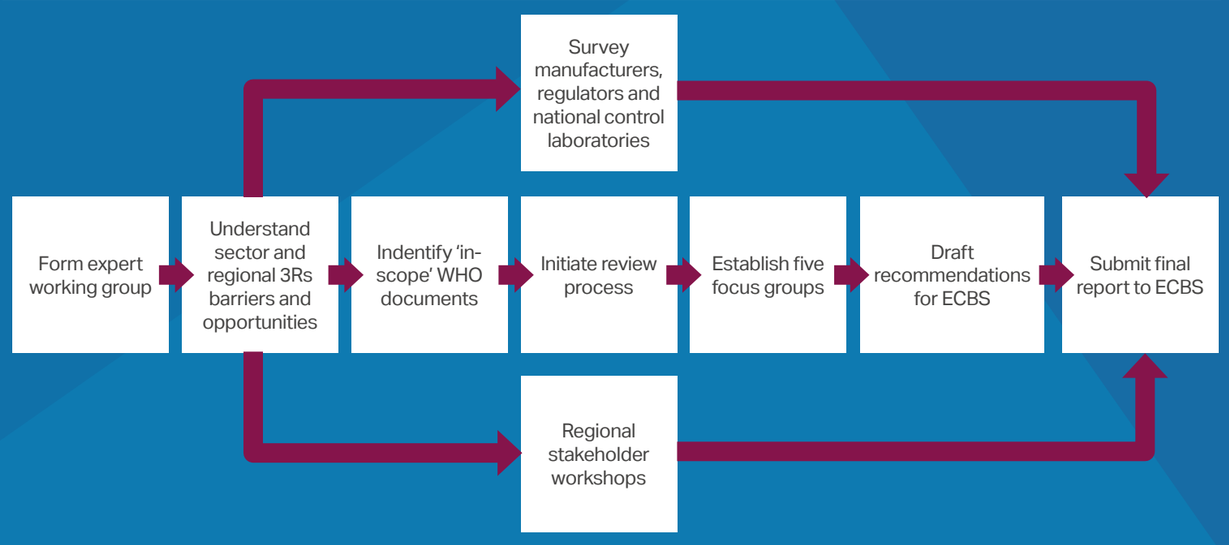

Figure 1. The process used to deliver the project.

Guideline review process

- An expert international working group of biologicals manufacturers, regulators and other relevant stakeholders was formed to support the project and review WHO guidelines for animal use requirements and existing 3Rs language (Table 1/Figure 2).
- Eighty-one WHO guidance documents were reviewed. Sixty-three described animal testing methods used to assess the presence of adventitious agents, neurovirulence, potency, pyrogenicity and toxicity of vaccines and biological products before they are released on the market.
- A focus group was established in each of these five areas to review the methods described and to propose alternative text/phrasing to those sections.
- Of 472 individual items found during review, 267 were 'in-scope' animal tests. Of these, 179 suggested non-animal alternatives or identified 3Rs approaches.
- The review also highlighted significant differences in the way specific assays are described across product guidelines and the extent to which 3Rs opportunities are recognised (Figure 3).

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

Table 1. The organisations represented on the working group.

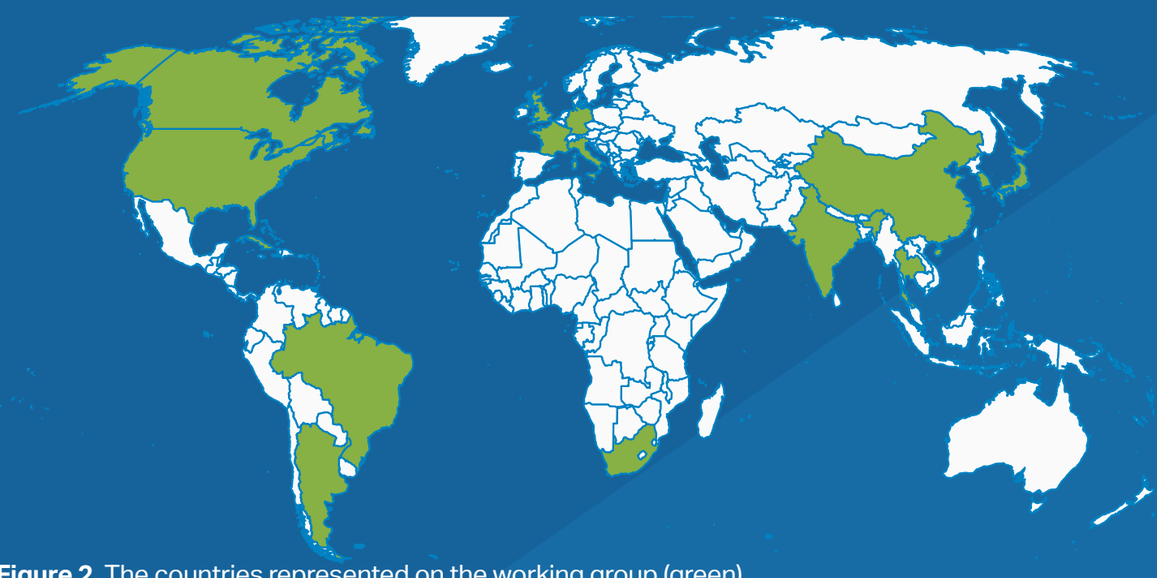

Figure 2. The countries represented on the working group (green).

3Rs language	Pyrogenicity/endotoxin testing
For ethical reasons, it is desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals in research, and consideration should be given to the use of appropriate *in vitro* alternative methods for safety evaluation.	A test that has been found to be suitable for the current vaccine involves injection into the ear vein of rabbits...
WHO has promoted the replacement of animals for experimental purposes, both for ethical reasons and in the interests of progressive improvement in product safety and quality.	Each final lot should be tested for pyrogenic substances. The test procedures should be approved by the national regulatory authority.
...attention should be paid to the care and handling of laboratory animals to minimize effects of environment and nutrition and to maximize efficacy in their use, particularly in the quality control of bacterial vaccines. Animals should be bred and maintained in such a way that the maximum possible standardization and reproducibility are obtained.	The vaccine in the final container should be tested for pyrogenic activity by intravenous injection into rabbits or by a Limulus amoebocyte lysate (LAL) test, which should be validated for this purpose.
Any scientist carrying out bioassays using animals should be aware of the 3Rs, as described by Russell and Burch (1959). Thus, *in vivo* bioassays should only be used if scientifically valid *in vitro* or other techniques are not available. Refinement should be introduced as far as possible in *in vivo* bioassays. For example, several of the assays described here employ 'humane endpoints'.	The endotoxin content of the final product should be determined using a suitable in vitro assay such as a LAL test. When required, the monocyte activation test (MAT) or rabbit pyrogenicity test may be used for monitoring potential pyrogenic activity subject to the agreement of the NRA.

Figure 3. Examples of language related to the 3Rs and pyrogenicity/endotoxin testing in current WHO guidelines.

Stakeholder engagement

Several distinct communities facilitate global vaccine and biological quality control and batch release testing, including manufacturers, NCLs and NRAs. There are subtle, but important, regional variations in approaches and opportunities to implement the 3Rs. To understand these better and ensure the recommendations to ECBS facilitate global uptake we engaged regularly with communities through:

- Regional workshops to focus on region-specific challenges. Workshops were hosted for Europe, Asia and Pan-America regions. Presentations were recorded and are available on the NC3Rs website [2].
- Surveying the manufacturer and regulatory communities to gather global information on the opportunities and barriers for these key stakeholder groups. The output of the manufacturer survey has been published in Biologicals [3]. The regulatory survey data has been submitted for publication.

These activities highlighted the global appetite to apply 3Rs approaches within quality control and batch release testing of biological products, and the need for greater integration of the 3Rs within WHO guidelines to support these aspirations. There was also considerable support for WHO to develop a general 3Rs guideline or position statement similar to chapter 5.2.14 in the European Pharmacopeia [4].

Next steps

The recommendations and project report have been submitted to WHO ECBS for consideration in October 2023. Implementation of the recommendations will be coordinated by WHO in a secondary phase of the project. The NC3Rs will continue to support this process and is developing a follow-on project to facilitate this.

References

1. Lilley E *et al.* (2021). Integrating 3Rs approaches in WHO guidelines for the batch release testing of biologicals. *Biologicals* 74: 24-27. doi:10.1016/j.biologicals.2021.10.002.

2. nc3rs.org.uk/3rs-resources/impact-3rs-approaches-quality-control-and-batch-release-testing-biologicals.

3. Lilley E *et al.* (2023) Integrating 3Rs approaches in WHO guidelines for the batch release testing of biologicals: Responses from a survey of vaccine and biological therapeutics manufacturers. *Biologicals* 81: 101660. doi:10.1016/j.biologicals.2022.11.002.

4. Council of Europe. 5.2.14 Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines. *European pharmacopoeia*. 2018; EDQM.

To learn more about this project please join our session: 'S409 – Warp-speed replacement? Remaining challenges in vaccine and biologics batch testing', Tuesday 29 August, 14.00 – 16.00. Visit our website: nc3rs.org.uk/WHOproject.