

**BIOLOGICAL REFERENCE STANDARDS FOR  
MULTIVALENT VACCINES  
QUALIFICATION STRATEGIES AND CHALLENGES  
FROM A NATIONAL CONTROL LAB PERSPECTIVE**

**CASS**

**BIOASSAYS 2020: SCIENTIFIC APPROACHES & REGULATORY STRATEGIES  
SESSION ON TECHNICAL ASPECTS OF BIOASSAYS THROUGHOUT THE PRODUCT**

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# Legal Framework



Council Directive 2001/83/EC, amended by Directive 2004/27/EC, formerly Council Directives 89/342/EEC and 89/381/EEC.

**Article 114** of the codified Directive relating to medicinal products for human use, allows but does not require a Member State laboratory to test a batch of an immunological medicinal product or a medicinal product derived from human blood or plasma before it can be marketed.

In Europe, 100% of the batches are tested by an Official Medicines Control Lab before being marketed (roughly 1250 batches per year in our lab).

# Biological Reference Standards for multi-valent vaccines

## Biological Reference Materials

Potency testing:

- **biological reference vaccines** (*in vivo* and *in vitro*)
- **biological reference standards** (*in vitro*)

Validity of assay:

- **biological controls** (*in vitro*)

# Biological Reference Standards for multi-valent vaccines

Some compendial reference standards are available through EDQM / WHO:

- Reference vaccines: BRP3 (Tetanus), BRP4 (Diphtheria) for challenge tests
- Reference standard: Bordetella pertussis mouse antiserum BRP batch 2 for serology tests on mice

# Biological Reference Standards for multi-valent vaccines

Manufacturers prefer in-house reference standards for the following reasons:

- homologous reference
- representative of own production
- easier to manage (supply, qualification, bridging schedule)

But mandatory to qualify in-house reference standard *versus* the International reference standards and to monitor consistency of results overtime

# Biological Reference Standards for multi-valent vaccines

From the OMCL point of view:

Compendial reference standards are:

- easier to manage (single bridging study)
- products from different manufacturers can be analysed in the same run, with reduced use of animals
- International standards are qualified through collaborative studies (EDQM / WHO)
- Same units for each user

# Biological Reference Standards for multi-valent vaccines

From the OMCL point of view: (cont')

## Non-compendial reference standards:

- one reference vaccine for each product (e.g. aP)
- increased use of animals for routine tests and bridging studies
- Subject to more variability (lack of qualification by collaborative studies)
- no comparability between manufacturers (different units)
- Less assured continued availability

# Biological Reference Standards and Bridging Studies

- A **switch** from one reference standard to another may lead to a **shift** in the results obtained, therefore bridging study is required
- In any bridging study, **influences** due to other factors (e.g. assay reagents or materials) should be evaluated
- Changes of reference material should be **anticipated** in order to facilitate qualification and **continuity** of routine testing results from an OMCL perspective



# Biological Reference Standards

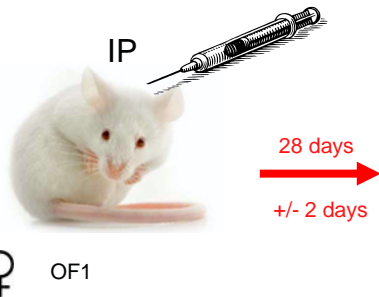
- For the **bridging of controls**, the data obtained (e.g. mean, coefficient of variation) are evaluated (control chart) to keep previous **limits** of acceptability or **define new limits**
- Apply **manufacturer's control limits** in the OMCL control charts e.g. if the **same method** is used and no indication of **systematic** differences at the OMCL

Refer to :

- [https://www.edqm.eu/sites/default/files/omcl-handling-and-use-noncompendial\\_reference\\_standards.pdf](https://www.edqm.eu/sites/default/files/omcl-handling-and-use-noncompendial_reference_standards.pdf) for further guidance (OMCL guidelines)
- Recommendations for the preparation, characterization and establishment of international and other biological reference standards (WHO TRS 932, 2006)

# Acellular pertussis test design: SEROLOGY

Immunization

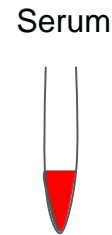


28 days  
+/- 2 days

Bleeding



Centrifugation



ELISA



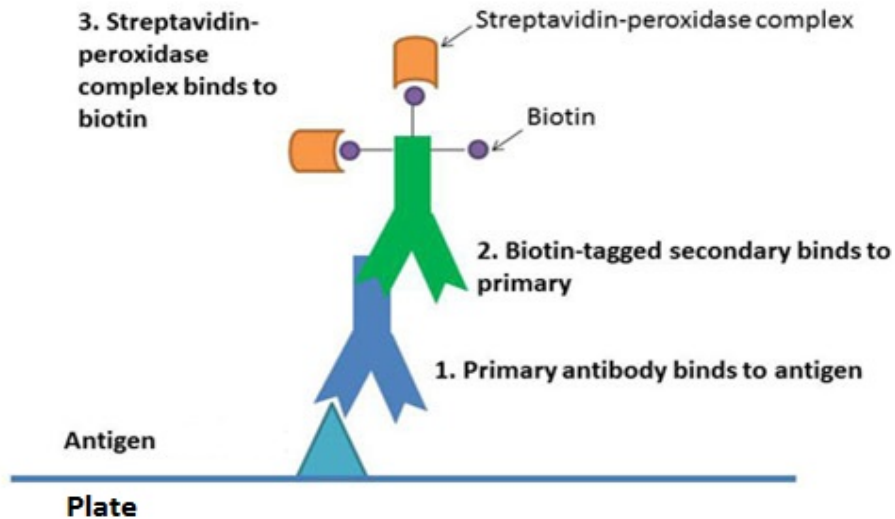
1 dilutions of vaccine and **reference** - 10 animals / dilution  
Negative mice - 5 animals

Calculations: based on geometric mean of 10 values then Relative potency or no significant difference between vaccine and reference vaccine

The **first reference vaccine** is usually a lot used in clinical trials.  
The bridging of the reference vaccine is thus of high importance !

# Biological Reference Standards

## ELISA Pertussis Immunogenicity Testing



A few words about the ELISA test. First, antigens are coated on the plate. Then the primary antibody from mouse serum binds to the coated antigen. The test uses then secondary biotin-tagged antibodies. The detection is amplified with streptavidin-peroxidase complex which strongly binds to the biotin. The peroxidase can then convert the substrate which shows a colour change depending on antibody concentration.

# Biological Reference Standards

Biological standards are often used to **ensure traceability** to the **first clinical lot**

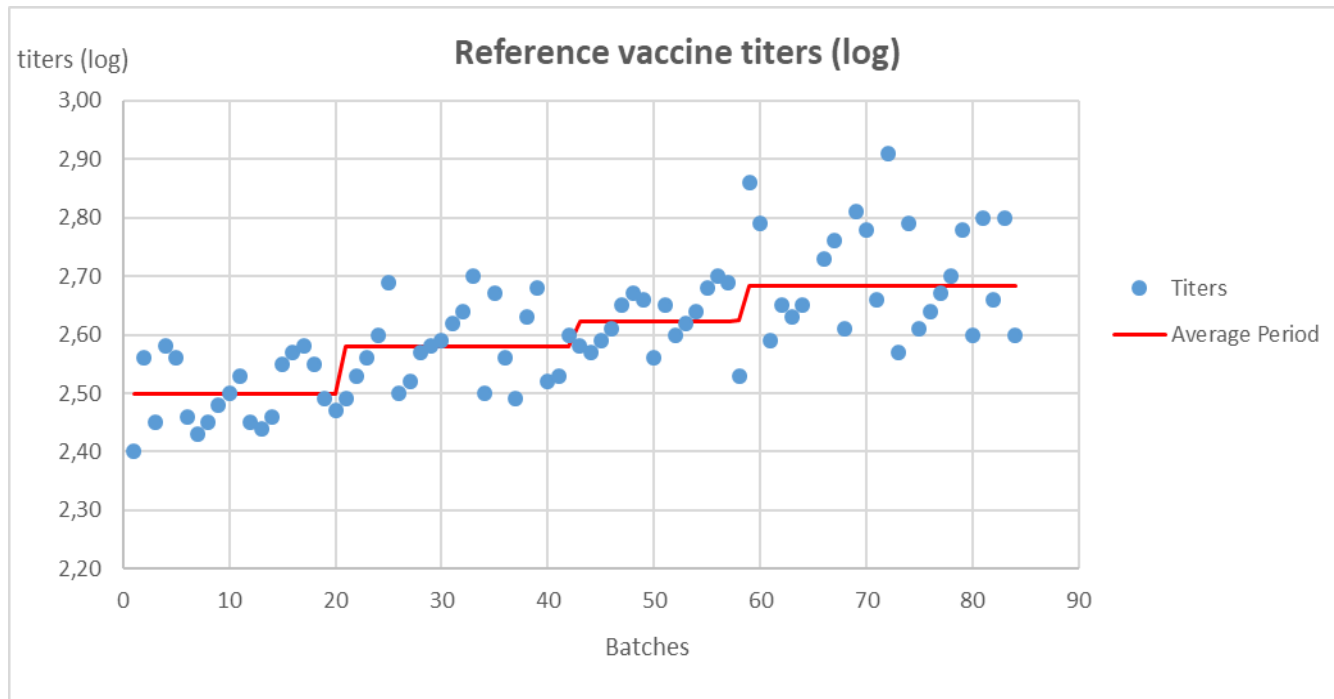
Potential strategies:

- Bridging study *versus* primary
- Successive bridging studies to align test results over time (with or without determination of **correction factors**)

# Biological Reference Standards

## Case study

How to handle such a case?: upward drift from the manufacturer's point of view but no drift for the OMCL



# Biological Reference Standards

It is **strongly recommended** to communicate in an appropriate and timely manner with the manufacturer to avoid shortage of reagents and materials and facilitate smooth performance of bridging studies

New Manufacturer Reference (shelf-life 3-5 years)

Bridging and lifetime use within OMCL

# Biological Reference Standards

New Manufacturer Reference (shelf-life 3-5 years)

Bridging and lifetime use within OMCL

## Risks:

- due to gaps (i.e. time and stability trends), the results between OMCL and manufacturer may be significantly different
- increased workload, due to lack of time/material to qualify new reference

# Biological Reference Standards: Future challenges for an OMCL

- Serology assays with multiplex technology (Luminex<sup>®</sup>, Meso-Scale<sup>®</sup>,...): implies increase use of in-house standards and related workload
- Use of GMU specification instead of the use of a reference vaccine should limit the complexity of bridging studies
- Move from *in vivo test* to *in vitro test* (3R's regulation, Vac2Vac IMI project): should limit use of animals, testing variability and discrepant results between manufacturers and OMCLs but will increase the need to select and qualify new international or in-house standards



# Biological Reference Standards: Future challenges for an OMCL

- More complex vaccines (2 to 5 components for pertussis, 15-valent pneumococcal vaccines need a reference standard and biological control for total polysaccharide content, free polysaccharide content !)
- Different testing procedures and specifications between OMCL and manufacturers

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