IMPLEMENTING THE 3RS IN QUALITY CONTROL AND BATCH RELEASE TESTING – VIEW FROM THE BELGIAN OMCL

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Quality of Vaccines and Blood Products
Sciensano

Outline

- Background: OMCL
  - Mission
  - Core activities
  - General regulatory framework
- Implementation of alternative methods in practice
- Other perspectives and future challenges
- Conclusions

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Background: Our Mission as an OMCL
(Official Medicines Control Laboratory)

We ensure the availability of vaccines, and blood products of assured quality to the human population and animals, at the national and international level by the implementation of analytical methods complying with the highest quality standards.

We provide scientific advice for biological medicinal products to the competent authorities and share our technical and regulatory expertise with both internal and external partners in research projects and capacity-building activities.

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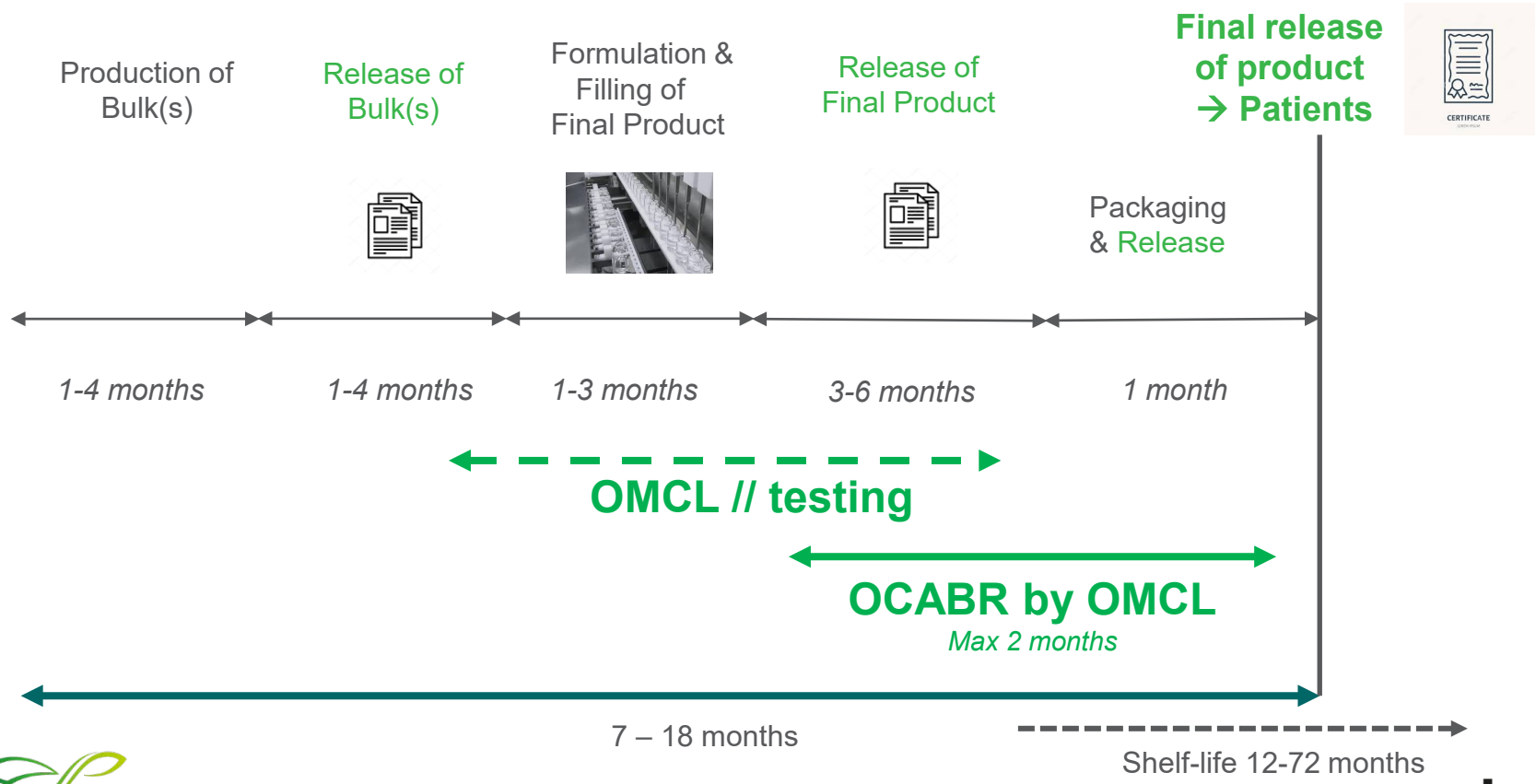
Background: Our Mission as an OMCL

- Vaccine batch release : from production to patients



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Vaccine batch release

>30 different vaccines released – 6 vaccine manufacturers

- Nearly **600 million** doses released by Sciensano in 2023 (2263 batches);
  >1.8 billion doses released at the pandemic peak.

- EU market (or non-EU countries requesting an EU certificate)
  → critical protocol review (OCABR* Product specific guidelines) **+ *in vitro/in vivo* TESTING**

- Non-EU market (WHO TRS)
  → critical protocol review + 20% testing at random

Among these products:

→ **9 DTaP vaccines requiring potency assays (*in vivo* testing)**

→ **13 vaccines for which endotoxin detection assays are performed (pyrogens)**

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*Official Control Authority Batch Release

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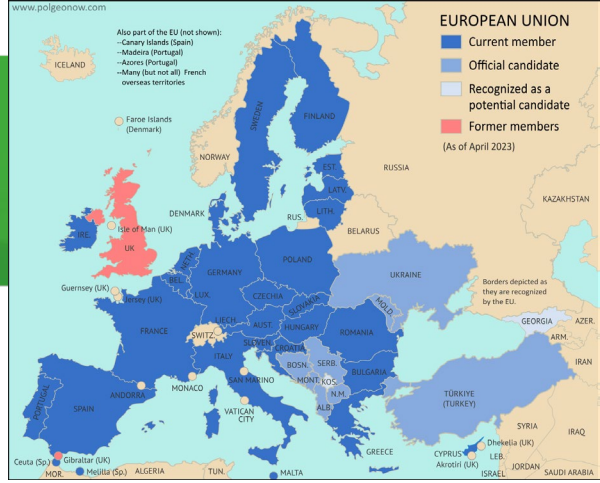
Background: General regulatory framework



European Pharmacopoeia

OCABR/OMCL guidelines

Regulate + improve the application of the 3R principles in animal testing

Directive 2010/63/EU

3RsWP

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Directive 2010/63/EU
→ European commission

- **Directive on the protection of animals used for scientific purposes**

- ✓ Fully applicable to regulatory testing of human and veterinary medicinal products

- ✓ **Encourages active implementation of 3Rs** : death should be avoided whenever possible / limited to a min. number of animals + limit stress/suffering/distress as much as possible + a method is not used if an animal-free alternative is recognized under national legislation

- ✓ Projects subjected to review by ethical committee

- ✓ New structures dedicated to the validation and regulatory acceptance of alternative testing methods

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EDQM (European Directorate for the Quality of Medicines and Healthcare)

- **EDQM particularly involved in the application of 3R guidelines:**

✓ Oversees the **OMCL network** in Europe:

→ Network for Official Control Authority Batch Release (OCABR) for human biologicals + Veterinary Batch Release Network (VBRN) for the veterinary biologicals

✓ Elaboration of **European Pharmacopoeia** (11th edition, Jan 2023) = primary source of official quality standards for medicines and their ingredients in Europe

→ Chapter 5.2.14 = Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines

✓ **Biological Standardization Program** (BSP) : aims at validating new pharmacopeial methods and establishing Ph. Eur. reference preparations for the QC of biological medicines

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OCABR/OMCL guidelines (EDQM)

- **3R statement for Administrative Procedure**

PA/PH/OMCL (13) 27 R: included in EU Administrative Procedure for OCABR (2013)

→ Revised to include an update to the annual report model (Annex V) and editorial revisions to Annexes IIb, IIf, III and IV under PA/PH/OMCL (14) 55 DEF;

- **3R Issues For Method Validation and Maintenance of Competence**

PA/PH/OMCL (12) 126 3R

→ Method validation: animal testing should be minimized + maintenance of competence of *in vivo* methods → animal testing should be avoided.

OCABR/OMCL guidelines (EDQM)

- **Mechanism for reducing in vivo testing by OMCLs during batch release**

PA/PH/OMCL (10) 48 R3 & specified in the product specific OCABR guidelines;

→ Implementation of reduction schemes (Overview of active schemes: Product and OMCL specific: PA/PH/OMCL (08) 12 4R)

- **OOS and 3R Special considerations for animal testing**

PA/PH/OMCL (14) 92 R2 (ANNEX 3.4 of the OMCL Network Guideline "Evaluation and Reporting of Results“)

→ Verification of OOS results generated during OCABR testing (taking the trends in manufacturer & OMCL data into account, if any)

3RsWP (prev. J3RsWG / JEG3Rs) (EMA)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

- The European Medicines Agency (EMA) is responsible for the scientific evaluation, supervision and safety monitoring of medicines in the European Union.

- **3RsWP** = joint expert working party on 3Rs

→ **see Sonja's presentation** *"Advancing the 3Rs at the EMA: a journey from the Joint Expert Group on 3Rs to the new 3Rs Working Party"*

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NC3Rs : reviewing animal use requirements in WHO biologics guidelines

- **Objective:** To review the animal test methods recommended for the lot release of biologics and vaccines which are described in WHO guidance documents and to identify opportunities for the integration of the 3Rs.

→ **https://nc3rs.org.uk/WHO-guidelines-review**

- Led by the UK National Centre for the 3Rs (**NC3Rs**) and co-funded by the Bill & Melinda Gates Foundation.

- Final report submitted to WHO/ECBS October 2023 including the **following recommendations**:

✓ Table of all animal tests referenced in each TRS with current and recommended new text incorporating 3Rs methods;

✓ Proposal for a standalone guidance on pyrogenicity/endotoxin testing;

✓ Proposal for a general 3Rs statement/guidance from WHO to support their adoption;

✓ Recommendations for ECBS on TRS stewardship to improve their utility and ability to be updated easily.

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NC3Rs : reviewing animal use requirements in WHO biologics guidelines

- **Interim response from WHO ECBS:**

> Animal testing approaches have long been used for the quality control and lot release of many vaccines and biotherapeutics. However, animal-based assays are inherently variable and highly time consuming, potentially causing delays in the availability of life-saving products. In light of recent significant advances in the implementation of non-animal technologies for the quality control of biological medicines, WHO had commissioned an independent review of the animal-based methods currently recommended in its written standards for biologicals. This review had been conducted by the National Centre for the 3Rs in the United Kingdom and its final report and associated proposals were presented to the ECBS. Acknowledging the quality and comprehensiveness of the work undertaken during the review, the ECBS recommended that a working group be established to build upon its findings and to develop further WHO guidance in this area.

https://www.who.int/publications/m/item/78th-ecbs-meeting-october-2023

- Full response expected by end of Q1 2024

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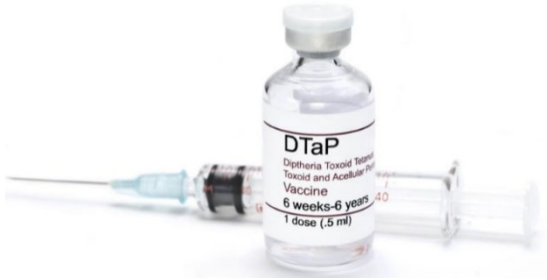
Implementation of alternative methods in practice → DTaP vaccines

- Category = detoxified adjuvanted vaccines
- Classified as « old » vaccines
  → Developed in the 1930s' and authorized in the 50s'
- Several combinations of antigens
  → Diphtheria, Tetanus, Pertussis (+ IPV and/or HepB)
- Confer active immunity against Diphtheria, Tetanus and Pertussis
- Limited alternatives to *in vivo* testing for potency assessment



Reduce/Refine/Replace

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In vivo testing: method principle

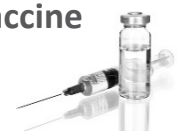
- **Diphtheria & Tetanus toxin challenges**

Day 0	→	Day 28	→	Day 29 to 32

Mice (T)
Guinea Pigs (D)
Vaccination

SC injection of

Reference vaccine
Tested vaccine



Lethal Challenge

SC Injection of
Toxin solution

Daily observation
→ Humane endpoints
Dead animals count

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Implementation of alternative methods in practice → DTaP vaccines

➢ Example 1: Implementation of reduction schemes

Cf. PA/PH/OMCL (10) 48 R3, 2017: Mechanism for reducing in vivo testing by OMCLs during batch release

	2018	2019	2020	2021	2022	2023
aP tests*	258	206	204	170	162	175
Certificates released	737	624	511	602	776	479
% Batches tested**	**35%**	**33%**	**40%**	**28%**	**21%**	**37%**
DT tests	205	89	124	108	95	83
DT testing/aP testing (%)***	**79%**	**43%**	**61%**	**64%**	**59%**	**47%**

Depending on the vaccine, the reduction scheme proposes to test:

- **only a percentage of final bulks/lots** (e.g. 20% for Diphtheria & 10% for Tetanus) issued from a given toxoid concentrate;

→ To insure maintenance of expertise: commitment to perform **≥3 SDAs and 1 MDA every year**.

- **only the first final bulk** formulated with the same batch of DT concentrate.

→ To insure maintenance of expertise, commitment to perform **≥2 potency assays/vaccine every year**.

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**No reduction scheme applied for aP serology*
***Tests performed on bulks while certificates released per lot*
**** Thanks to RS, further reduction of DT testing by up to 57%*

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Implementation of alternative methods in practice → DTaP vaccines

- **Example 2: Transition to single dilution assays (SDA)**

Multiple Dilutions Assay	Single Dilution Assay
* 3 or 4 dilutions / reference * 3 or 4 dilutions / tested vaccine * 12, 15 or 16 animals/dilution * Challenge Dose Control: 5 animals/test * Toxin activity Control: each test 5 animals & 3 dilutions * Calculations ED50 & LD50 determination * Results Potency in IU/Dose	* 1 Dilution / reference * 1 Dilution / tested vaccine * 12, 15 or 16 animals/dilution * Challenge Dose Control: 2 times/year 5 animals * Toxin activity Control: 2 times/year 5 animals & 3 dilutions * Calculations Fisher's probability test * Results PASS / FAIL

Decreasing the number of animals used by ~80%

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Implementation of alternative methods in practice → DTaP vaccines

- **Example 2: Transition to single dilution assays (SDA)**

Vaccine	Content	Testing
A	DTaP	MDA for D + **SDA** for T
B	DTaP + polio	MDA for D + **SDA** for T
C	DTaP + polio + HepB	**SDA**
D	DTaP + polio + HepB + Hib	**SDA**
E	DTaP	**SDA**
F	DTaP + polio	**SDA**
G	DTaP + polio	**SDA** for D + MDA for T
H	DTaP + polio + HepB + Hib	**SDA**
I	DTaP + polio + HepB + Hib	**SDA**

Implementation of alternative methods in practice → DTaP vaccines

- Example 3: Implementation of DTP serology

Day 0 → **Day 28**

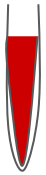
Mice
Vaccination

IP injection of
Tested vaccine/
Reference vaccine

Negative control = saline

Bleeding

BLOOD



O/N at Room t°
Centrifugation

SERUM

ELISA

Antibody titer
(mean of 10 mice = geomean)

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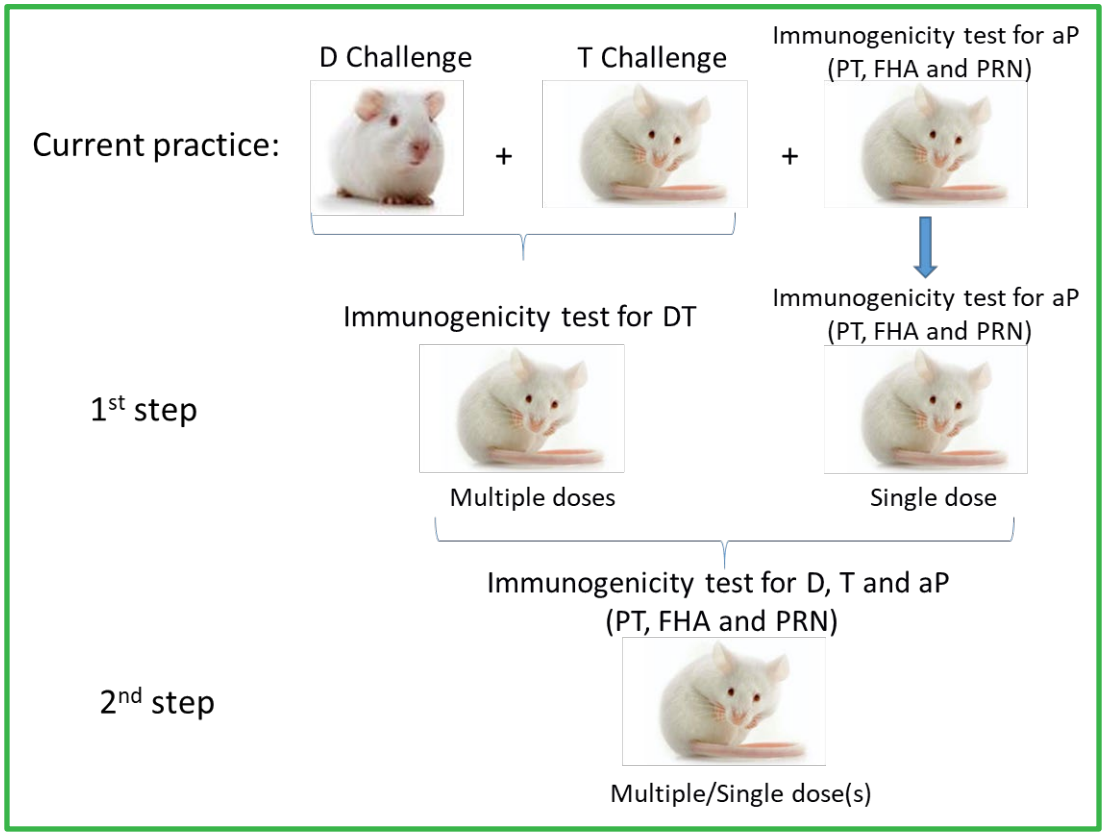
Implementation of alternative methods in practice → DTaP vaccines

- **Example 3: Implementation of DTP serology**



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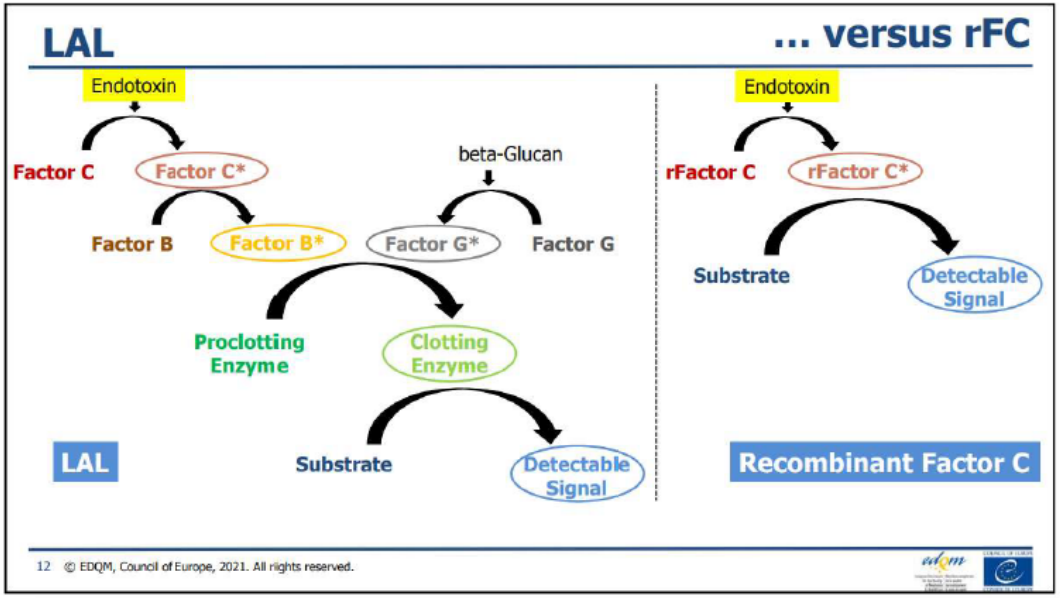
Implementation of alternative methods in practice → Detection of endotoxins

➢ **Example 4: rFC (recombinant Factor C)**

→ Alternative to the rabbit pyrogen test and the LAL test (Limulus amebocyte lysate)

→ Detection of endotoxins = pyrogen components of the exterior cell wall of gram-negative bacteria (fever-causing agents → highly limited concentration in vaccines)

Principle



LAL :

- Colorimetric kinetic detection method
- Use of horseshoe crab (population decreasing !)
- Large lot/lot variation
- Interference of beta-glucan
- Log/log correlation (time/concentration)

rFC:

- Fluorometric endpoint detection method
- Animal-free
- Smaller lot/lot variation
- No beta-glucan interference
- Log/log correlation (ΔRFU/concentration)

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Other perspectives and future challenges

➢ **Improvements for DT serology**

- Single dilution assay
- Reduction scheme
- Complete transition to DT+P serology

➢ **Validation of Multiplex assays (*in vitro*) cf. VAC2VAC project**

→ Animal-free + saving time and costs

→ 1 run to assess all antigens

→ Lower variability than *in vivo* methods (5-10% vs 30-50%)

	Number of animals for *in-vivo* potency and serological assays		Number of days to perform the assays	
	In-vivo	*In-vitro*	*In-vivo*	*In-vitro*
Diphtheria	Up to 116*	0	± 30*	1
Tetanus	Up to 116*	0	± 30*	1
acellular Pertussis	25#	0	28#	1

**challenge assay*
#serological assay

Conclusions

OMCLs are continuously taking 3Rs priorities into account by

- Reducing the amount of animals used for testing (cf. reduction schemes, implementation of SDAs, DTP serology);
- Replacing *in vivo* methods by *in vitro* alternatives whenever possible (cf. rFC, Multiplex).

All the while **maintaining the highest quality standards** for vaccines & blood products before market release

Final goal : no animal testing needed by OMCL for vaccine batch release

ETA: ~5 years

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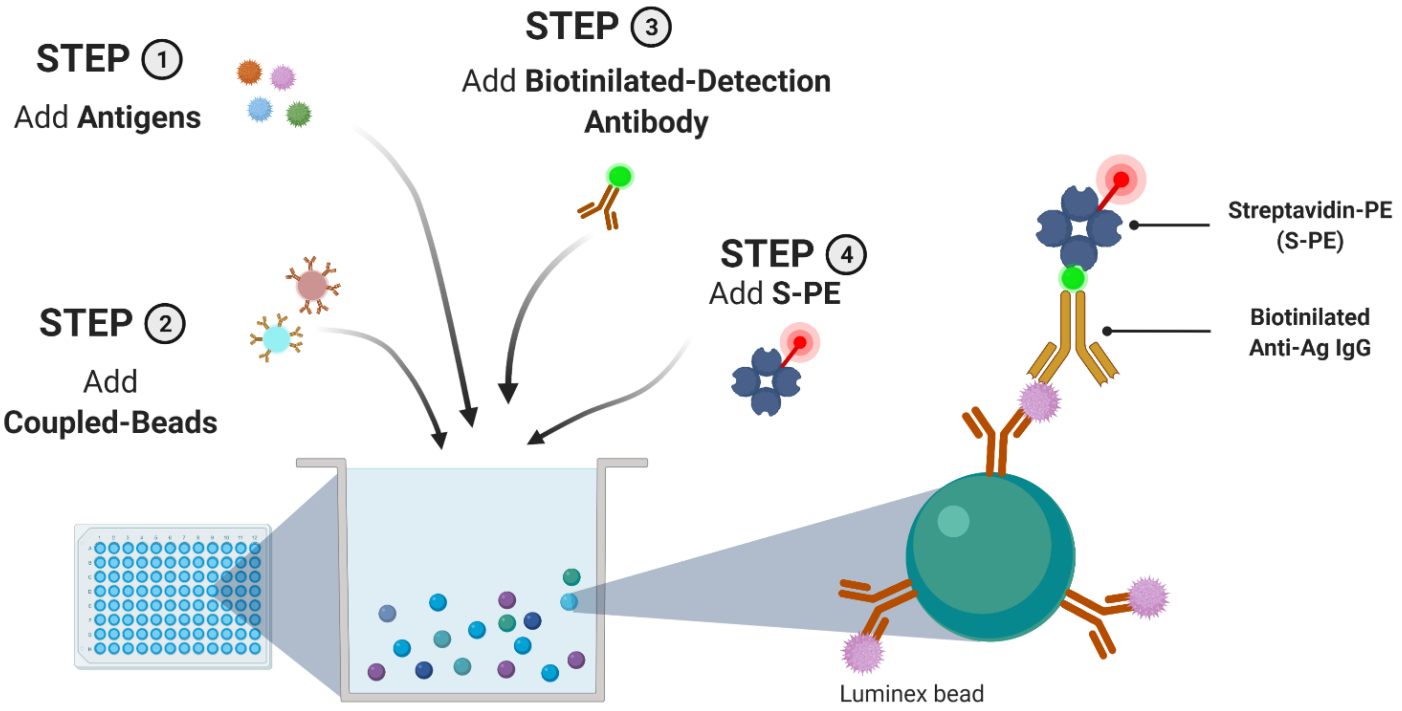
Reviewing animal use requirements in WHO biologics guidelines

https://nc3rs.org.uk/WHO-guidelines-review

Other perspectives and future challenges

- **Validation of Multiplex assays (*in vitro*)**

Method principle



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