DT CHALLENGE SINGLE DILUTION ASSAY: PROS AND CONS OF AN ANIMAL REDUCTION OPPORTUNITY – VIEW FROM THE BELGIAN OMCL

AFSA DTP WORKSHOP JAN. 30, 2025

Morgane Florens, PhD
Quality of Vaccines and Blood Products
Sciensano

Outline

- Background:
  - DTaP vaccines
  - The ‘old way’: MD challenge assays
  - SDA : Rationale & Guidelines

- Implementation of an SDA in practice
  - Conditions
  - Advantages
  - Limitations

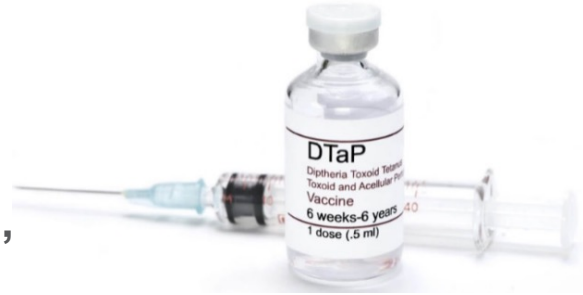
- Other perspectives:
  - DTaP serology
  - *In vitro* alternatives

sciensano

.be

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 and/or Hib)
- Confer active immunity against Diphtheria, Tetanus and Pertussis
- **Up to recently, limited alternatives to *in vivo* testing for potency assessment**

The ‘old way’: MD challenge assays

- Diphtheria & Tetanus toxin challenges

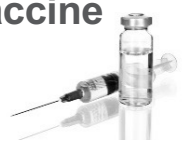
Day 0 → **Day 28** → **Day 29 to 32**

Mice (T)
Guinea Pigs (D)
Vaccination

Dunkin Hartley

SC injection of

Reference vaccine
Tested vaccine



Lethal Challenge

SC Injection of Toxin solution

Daily observation
→ Humane endpoints
Dead animals count

sciensano

.be

The ‘old way’: MDA challenge assays

- **Goal ?** To distinguish between potent and sub-potent products
- **How ?** By comparing the effective dose of reference and test vaccine

Day 0 → **Day 28** → **Day 29 to 32**

Vaccination — Lethal Challenge — Daily observation

Several dilution levels

Diphtheria / Tetanus

* 4 Dilutions / reference
* 4 Dilutions / tested vaccine
* 12 - 16 animals / dilution

* Toxin activity Control:
3 dilutions of the challenge dose with 5 animals

* Challenge Dose Control: 5 animals

Ref. vaccine dilutions

Date of Immunization	05-08-20
Test code	DIMU-20-06
Reference vaccine	Reference (BRP)
Dilution 1	1/16
Number of survivors/12	12
Dilution 2	1/32
Number of survivors/12	12
Dilution 3	1/64
Number of survivors/12	6
Dilution 4	1/128
Number of survivors/12	0

survivors

Test vaccine dilutions

Tested vaccine	Vaccine Type
Lot number	Vaccine lot
Dilution 1	1/20
Number of survivors/12	12
Dilution 2	1/40
Number of survivors/12	11
Dilution 3	1/80
Number of survivors/12	6
Dilution 4	1/160
Number of survivors/12	0

survivors

sciensano

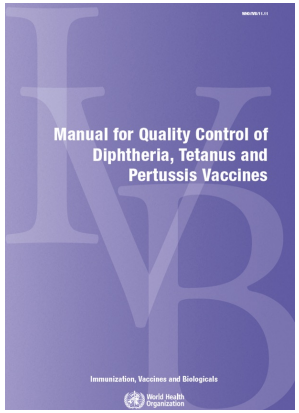
.be

Potency estimate (+ lower and upper fiducial limits)

Relevance of implementing an SDA

➢ **Context: 3R Principles – Replace, Reduce & Refine**

- As encouraged by WHO since 1980 and the EU Directive 2010/63/EU
- Target a drastic reduction in the use of laboratory animals
- Less animal suffering



➢ **Guidelines: When can we use an SDA?**

(WHO/IVB/11.11– Manual for Quality Control of DTP Vaccines)

- For a specific product which shows consistency in production and testing
- Adequate experience with MDA on a specific product
- With an adequate assay validation

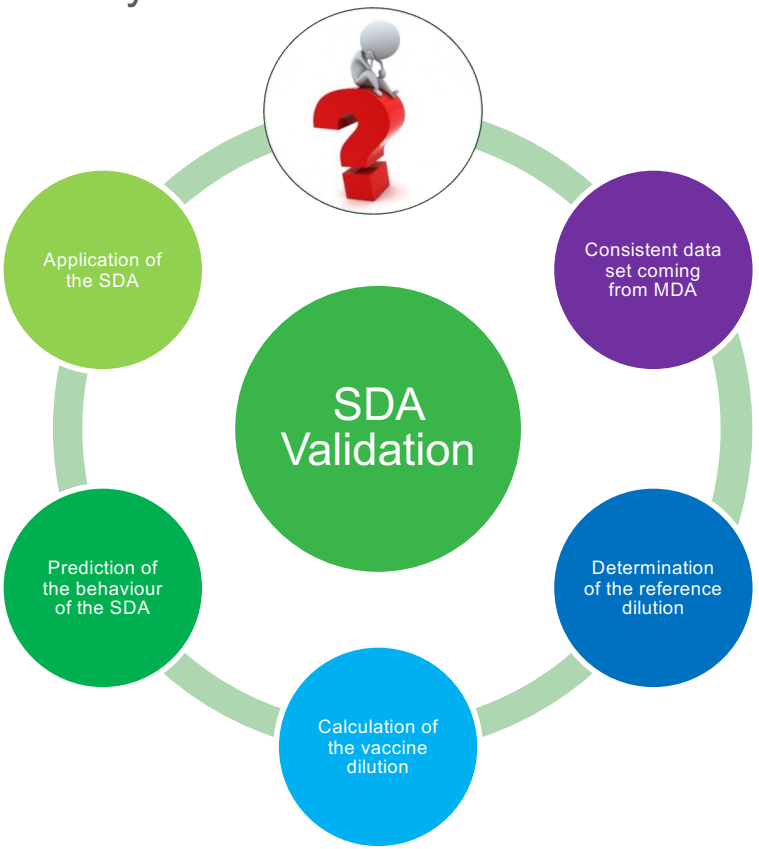
sciensano

.be

Implementation of an SDA in practice : Conditions

- **Adequate experience with MDA on a specific product**
  - Very good hands-on experience with the MDA assay (product specific)
  - Validated method (repeatability, reproducibility, robustness,…)
  - Evidence of good data consistency

- **Adequate SDA assay validation**



sciensano

.be

Implementation of an SDA in practice : Advantages

➢ **Implementation of the 3R principles**

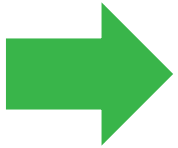
Multiple Dilutions Assay

* 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



Single Dilution Assay

* 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%

sciensano

.be

Implementation of an SDA in practice : Advantages

- **Other advantages**
  - Higher number of vaccines which can be tested in one run
  - Reduction of costs and resources
  - Less space required in the animal facilities
  - Saves time for the operators and the animal caretakers

Limitations and other perspectives

➢ Limitations

Necessity to have adequate experience with MDA on a specific product + good data consistency;

STILL AN *IN VIVO* METHOD = USING LAB ANIMALS

STILL A CHALLENGE ASSAY = INVOLVING ANIMAL SUFFERING

What are the alternatives?

sciensano

.be

Limitations and other perspectives: DTP serology

Day 0 → **Day 28**

OF1 ♀

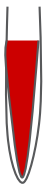
Mice
Vaccination

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)

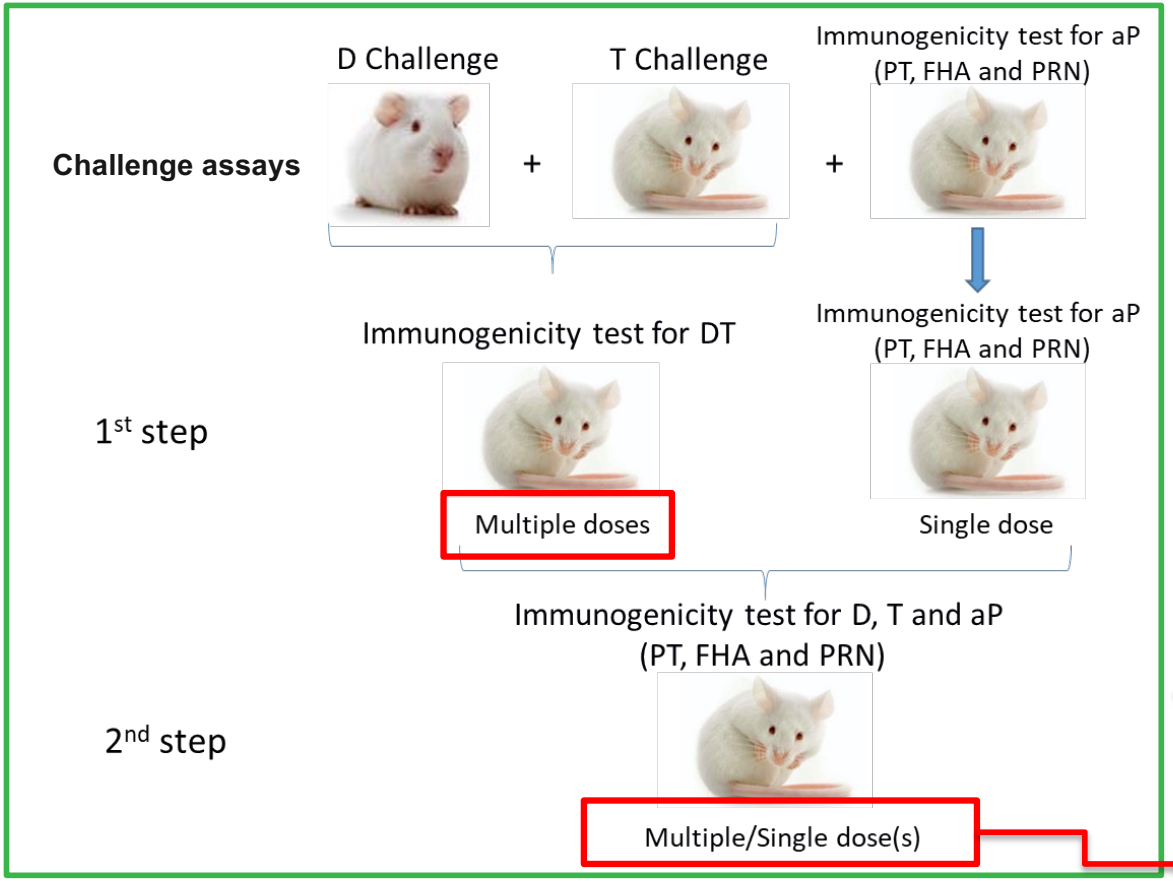
→ Results expressed as GMU

Mostly addressing animal SUFFERING compared to SDA challenge assays…

sciensano

.be

Limitations and other perspectives: DTP serology



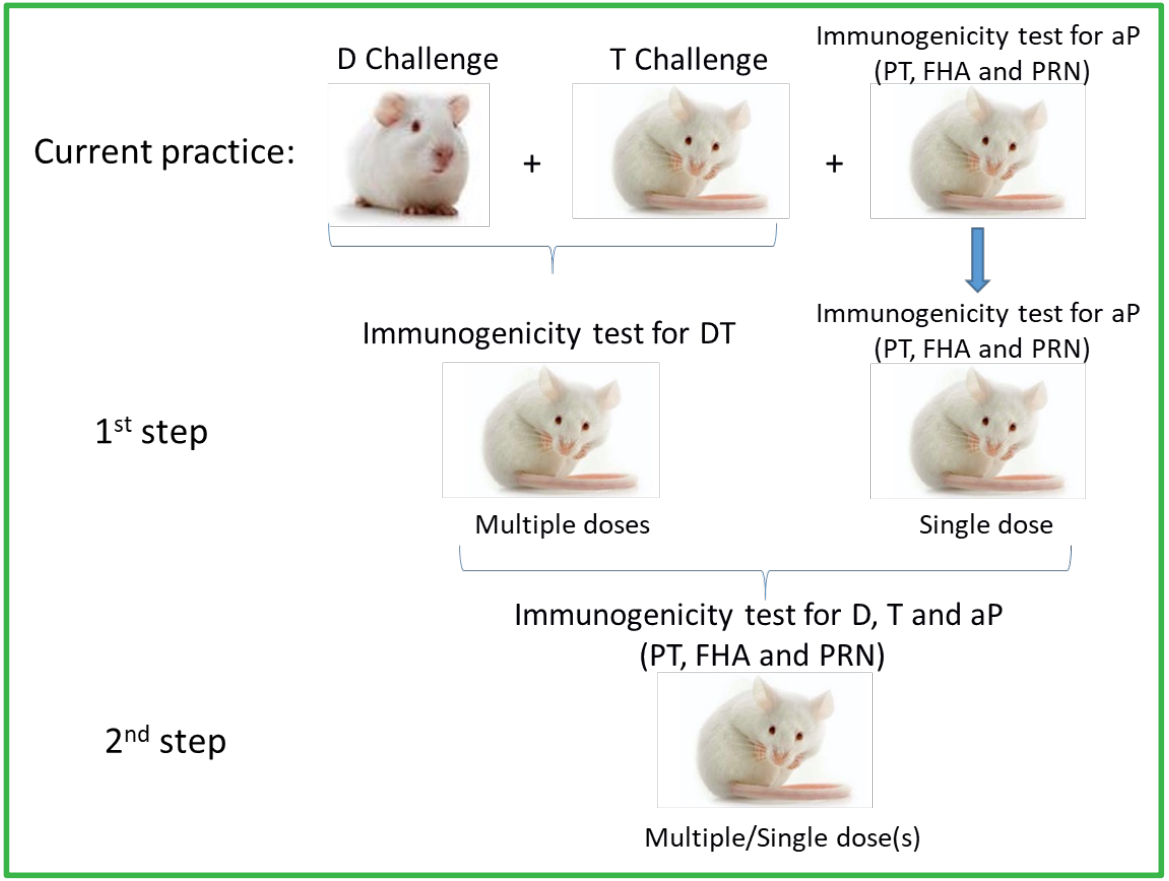
Progressively moving to one single batch of animals to test Di + Te + aP

Shifting to single dilution DTP serology to further decrease the number of animals used

sciensano

.be

Animal number decrease overview



DT MDA challenge assay + aP serology :

164 + 164 +35 = **363**

DT SDA challenge assay + aP serology:

36 + 36 +35 = **107**

DTP serology MDA:

135

DTP serology SDA:

45

sciensano

.be

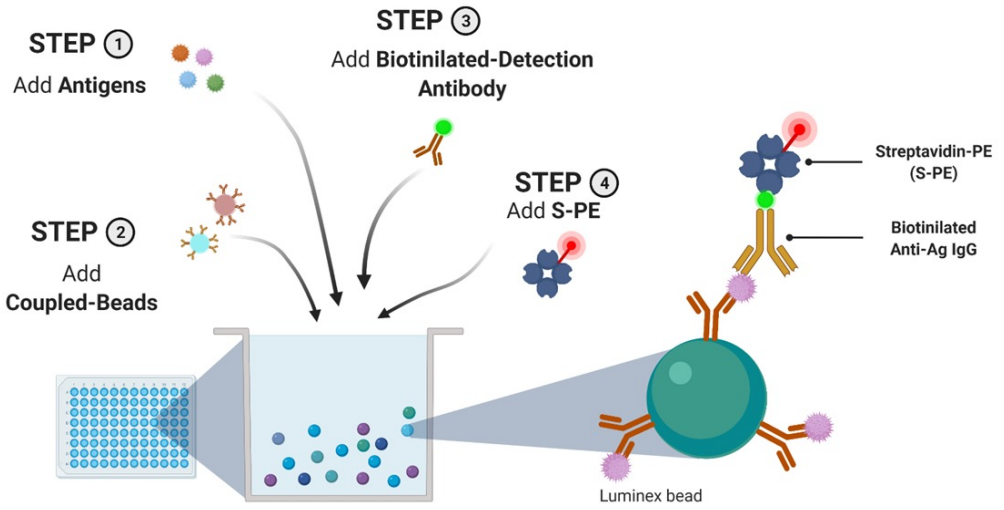
Limitations and other perspectives: *in vitro* alternatives

- **In vitro alternatives advantages**
  - **Animal-free** + saving time (±30 days to 1 day) and costs
  - 1 run to assess all antigens
  - Lower variability than *in vivo* methods (5-10% vs 30-50%)

Multiplex assays (cf. VAC2VAC project)

ELISA (cf. NIBSC: reagents available on demand)

See next presentations…



sciensano

.be

Conclusions

- **Advantages of SDA**

  - Drastic reduction in animal numbers
  - More vaccines tested in one run
  - Reduction of time, costs and resources
  - Less space required in the animal facilities

- **SDA limitations:**

  - PASS/FAIL read-out
  - Still an *in vivo* method
  - Still a toxin challenge

Further progress available with DTP serology and *in vitro* alternatives

sciensano

.be

sciensano

healthy all life long

Contact

Morgane Florens • <morgane.florens>@sciensano.be •
Fabrice Ribaucour • <fabrice.ribaucour>@sciensano.be •
Julien Auquier • <julien.auquier>@sciensano.be •

Sciensano • Rue Juliette Wytsmanstraat 14 • 1050 Brussels • Belgium
T +32 2 642 51 11 • T Press +32 2 642 54 20 • info@sciensano.be • www.sciensano.be

.be