

DTAP VACCINES : AN OVERVIEW OF INITIATIVES TO REDUCE ANIMAL SUFFERING IN HUMAN VACCINE BATCH RELEASE

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Quality of Vaccines and Blood Products
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Background: Our Mission

To ensure the availability for the population of vaccines and blood products of assured quality, on independent scientific basis and in an international context, using analytical methods validated by highly-skilled team and providing scientific advices for the biological medicinal products.

Background: Our Core Activities

- **Batch Release** of Biological Medicinal Products (vaccines & plasma-derived medicinal products), as OMCL (OMCL-B)
- **Advising** during licensing and GMP inspections for vaccines, Plasma derivatives, rDNA Biological Medicinal Products, Biosimilars
- **R&D projects** (e.g. EU IMI2 project incl. VAC2VAC)
- Ad hoc **regulatory activities** (Draft/revision of guidelines/monographs, audits, assessments, inspections)

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Vaccine batch release

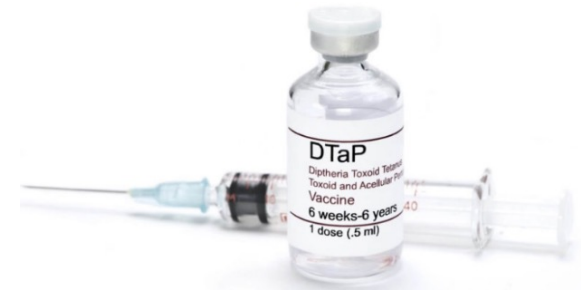
>30 different vaccines released – 6 vaccine manufacturers

- 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

Among these products: 9 DTaP vaccines relying on *in vivo* testing

DTaP vaccines

- Confer active immunity against Diphtheria, Tetanus and Pertussis
- 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)
- Limited alternatives to *in vivo* testing for potency assessment



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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

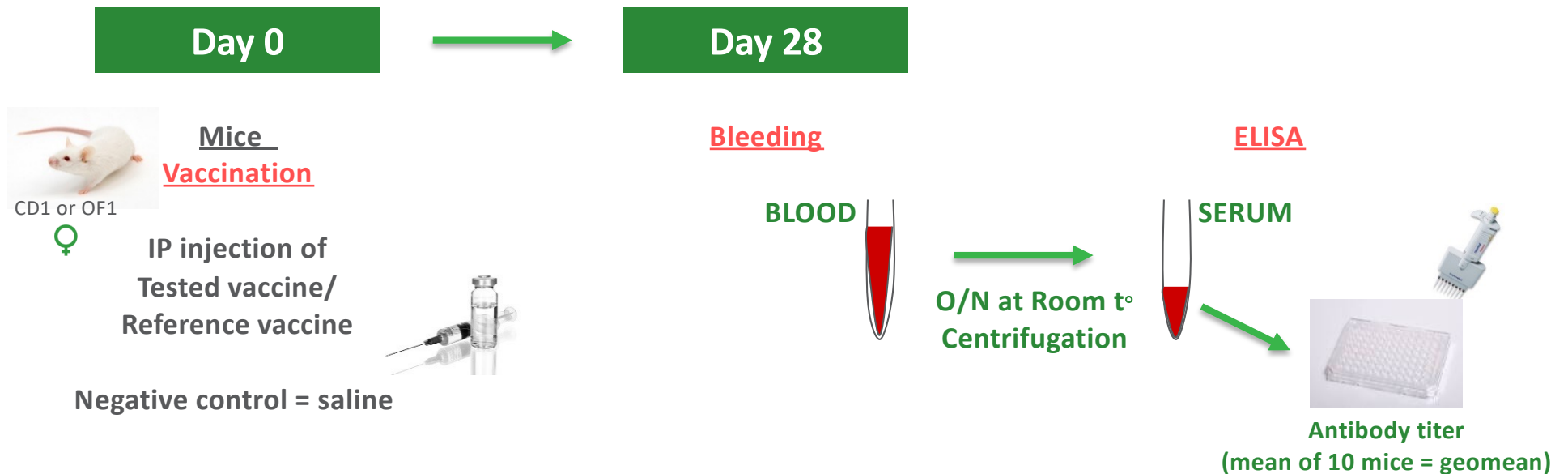


Daily observation
→ Humane endpoints
Dead animals count



In vivo testing: method principle

➤ aP serology



Comparison with a reference

- ➔ Relative potency (Vaccine titer / Reference titer) = $RP \approx 1$
- ➔ No statistical difference (T- Test: $p > 0.05$)

In vivo testing: Reduce / Refine / Replace

1) Reduce

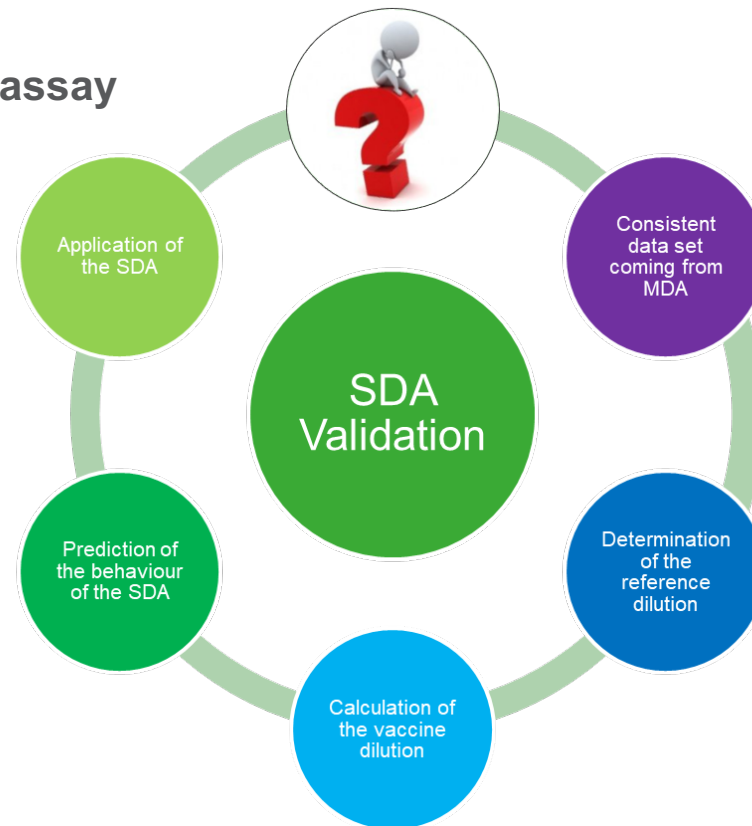
- Multiple dilution assay → Single dilution assay
- Reduction scheme



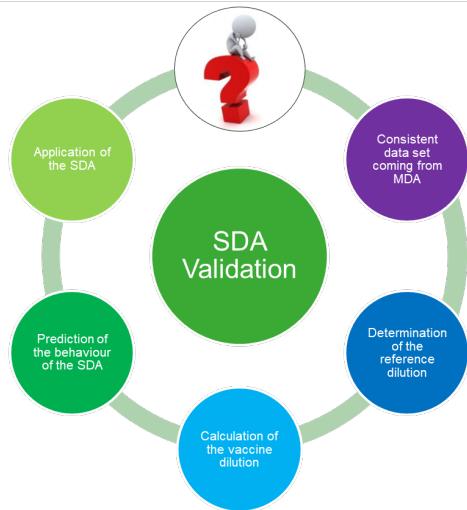
In vivo testing: Reduce / Refine / Replace

1) Reduce

- **Multiple dilution assay → Single dilution assay**
- Reduction scheme



In vivo testing: Reduce / Refine / Replace



➤ Consistent data set from MDA

- Good hands-on experience of MDA
- Validated method (repeatability, reproducibility, Robustness, etc.) + product specific
- Good data consistency (enough data + MDA produces reliable and consistent results)

➤ Determination of reference dilution

- average of 10-20% survival

➤ Calculation of vaccine dilution

- min. required dose compared to the dilution of the reference vaccine conferring 10% protection

➤ Prediction of behavior of SDA

- $\leq 10\%$ contradictory results between MDA results and prediction for SDA

In vivo testing: Reduce / Refine / Replace

1) Reduce

➤ Multiple dilution assay → Single dilution assay

➤ Reduction scheme

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

In vivo testing: Reduce / Refine / Replace

1) Reduce

- Multiple dilution assay → Single dilution assay

- **Reduction scheme** →

The scope of this reduction scheme is to reduce animal use for Diphtheria and Tetanus potency testing

In vivo testing: Reduce / Refine / Replace

1) Reduce

➤ Reduction scheme

The scope of this reduction scheme is to reduce animal use for Diphtheria and Tetanus potency testing

- ➔ Background for this proposal was the **PA/PH/OMCL (10) 48 R, 2010: Mechanism for reducing in vivo testing by OMCLs during batch release**
- ➔ For validation, potency data obtained on D and T components tested by the manufacturer and by the OMCL over several years were used.
- ➔ D and T potency were shown to be consistently and significantly higher than the specification limit (MDAs) or than the reference preparation (SDAs).

In vivo testing: Reduce / Refine / Replace

1) Reduce

➤ Reduction scheme

	2017	2018	2019	2020
Batches tested	574	258	203	181
Batches released	771	677	628	523

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

- **only a percentage of final bulks/lots** (e.g. 20% for Diphtheria and 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 a same batch of DT concentrate.
- ➔ In accordance with the OMCL guideline, commitment to perform **≥2 potency assays/year** on each vaccine to maintain testing proficiency.

In vivo testing: Reduce / Refine / Replace

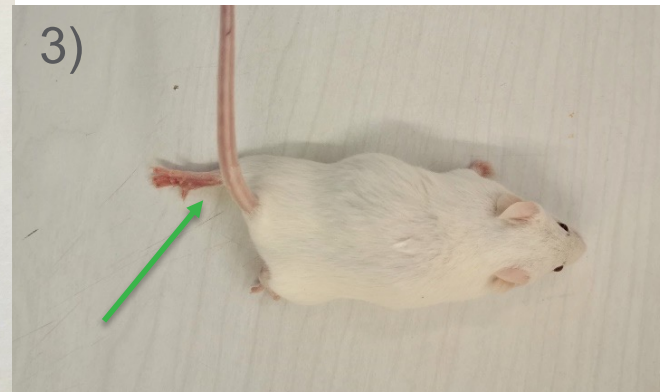
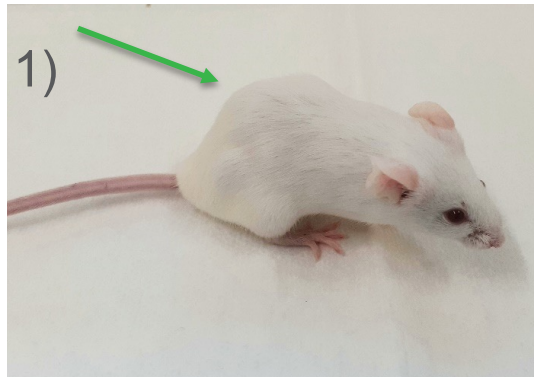
2) Refine

- Humane endpoints

In vivo testing: Reduce / Refine / Replace

2) Refine

- Humane endpoints: **MOUSE** → - First sign: “Hunchback” mouse
- Euthanasia in case of : full paralysis of back leg close to challenge site



In vivo testing: Reduce / Refine / Replace

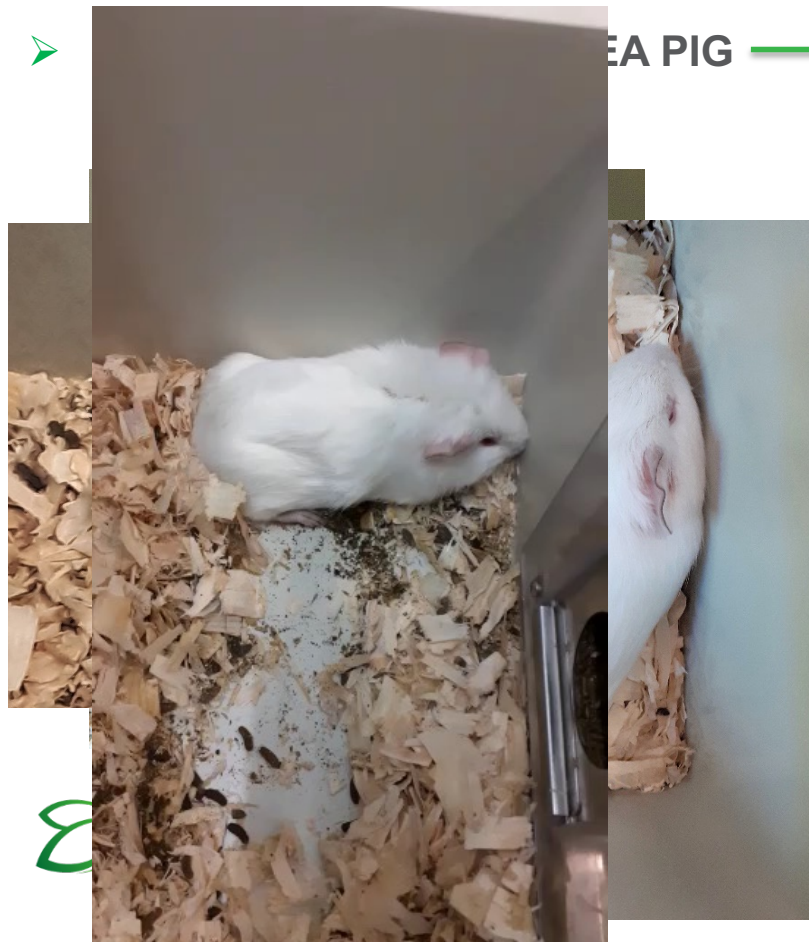
2) Refine

➤ Humane endpoints: **GUINEA PIG** → Symptoms assessed:

- Red circumference of eye
- Rough fur
- Oedema
- Hollowed flanks
- Exhaustion
- Apathy
- Difficulty in keeping head straight
- No response to external stimuli
- Difficulty to breathe

In vivo testing: Reduce / Refine / Replace

2) Refine



EA PIG → Symptoms assessed:

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→ Euthanasia if GP presenting several of them

In vivo testing: Reduce / Refine / Replace

3) Replace

➤ DT serology

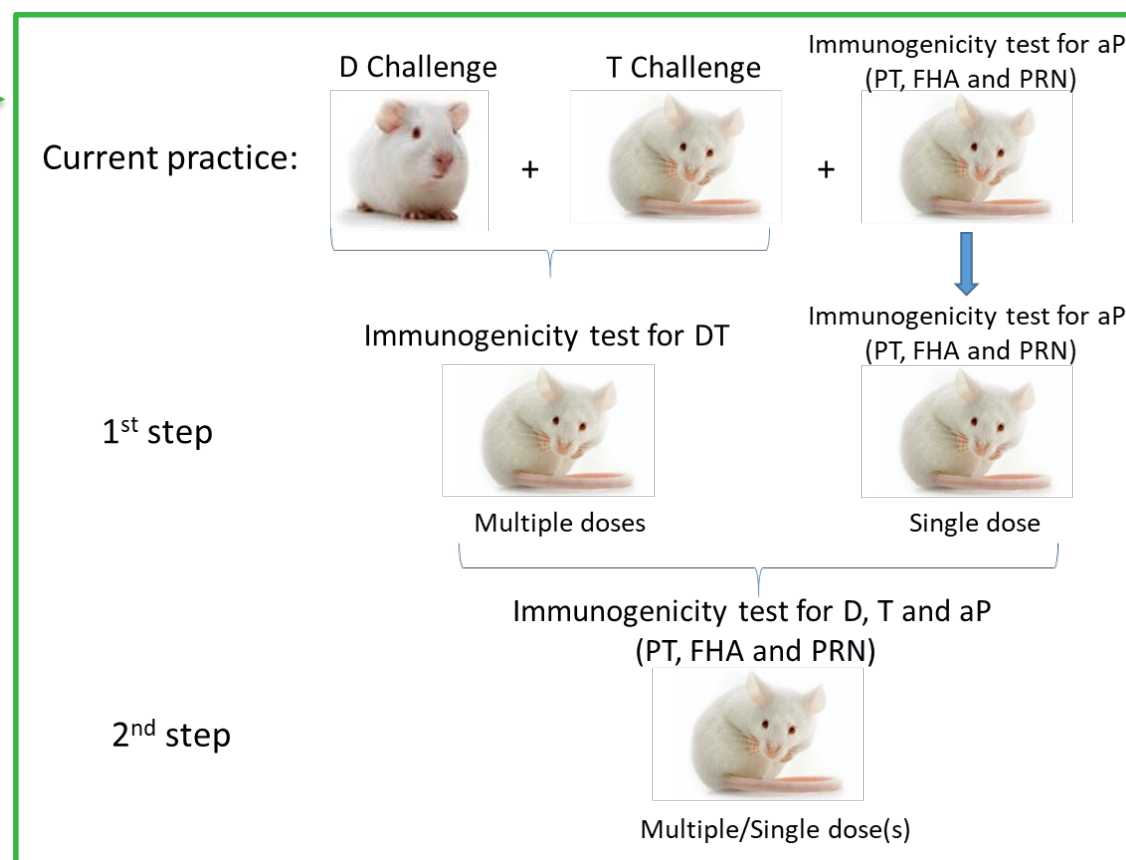
➤ VAC2VAC

In vivo testing: Reduce / Refine / Replace

3) Replace

➤ DT serology →

➤ VAC2VAC



In vivo testing: Reduce / Refine / Replace

3) Replace

➤ DT serology

➤ **VAC2VAC** → Demonstrate **proof of concept** of the **consistency approach** for batch release testing of established vaccines using sets of *in vitro* and analytical methods :



1. Development of new or optimisation of existing **non-animal methods** for consistency testing;
2. **Pre-validation** of selected methods;
3. **Regulatory acceptance** of the consistency approach.

Additional info VAC2VAC → What has and will be achieved for 3Rs

Rabies in vitro potency assay




































Strain-specific replacement ELISA have been designed. Validation ongoing / done / method filed (depending on manufacturer)

Completed: Substitute Rabbit pyrogen test for TBEV vaccine

National reference lab and Manufacturer validated the alternative. Triggered a discussion to revise the concerning Eu.Ph. monograph

Clostridium chauvoei in vitro potency assay

A promising replacement ELISA has been set up and will be transferred to manufacturers for validation and implementation

		Triggered a discussion to revise the code												
 Veterinary Human 		IBV	Leptospira	Rabies	Chauvoei*	Perfringens*	Quil A Adjuvant	Diphtheria	Tetanus*		Pertussis		TBEV	
		Potency Chicken Serology Challenge	Potency Hamsters Challenge	Potency Mice Challenge Serology	Potency Guinea Pigs Challenge	Safety Mice Detox		Potency Guinea Pigs Challenge Mice Serology	Safety Guinea Pigs Detox	Potency Mice Challenge Mice Serology Rabbits Serology	Safety Mice Detox	Potency Mice Serology	Safety Rabbits Pyrogen	Potency Mice Challenge
	WP 1 Physio-chemical									 				
	WP 2 Immuno-chemical							 						
	WP 3 Cell Based													
	WP 4 Bioinformatics													

Substitute mice challenge assay for TBEV vaccine with ELISAs

ELISAs for two TBEV vaccines qualified. Collaborative study in preparation among National reference lab and manufacturers

Clostridium perfringens C in vitro safety/toxin content assay

A substitution of the in vivo safety assays has been developed and transferred to manufacturers for further assessment and validation

Substitute in vivo potency assays for Diphtheria, Tetanus and Pertussis

Proof of concept achieved. Transfer of the methods to industry partners ongoing. Replacement of animal derived antibodies with monoclonal initiated

✗ Stopped. Assays are no longer compendial acc. to the European Pharmacopeia

TAKE-HOME MESSAGE



- Primary mission as an OMCL = **ensure the availability for the population of vaccines of assured quality** based on critical protocol review and independent vaccine batch testing → still partly relying on *in vivo* methods with regards to **DTaP vaccines**.
- For ethical reasons, several initiatives have been developed / are under development within our group in order to better implement the 3R principles, i.e. to **reduce/refine/replace the use of lab animals** as much as possible.
- These initiatives mainly consist in:
 - **Reducing** the number of tests performed via the implementation of a **reduction scheme**,
 - **Reducing** the number of animals used by shifting to **SDAs** instead of MDAs,
 - **Refining** our assessment of animal suffering using carefully selected **humane endpoints**,
 - Progressively **replacing** toxin challenge tests by **serology and *in vitro* alternatives**.

ACKNOWLEDGEMENTS

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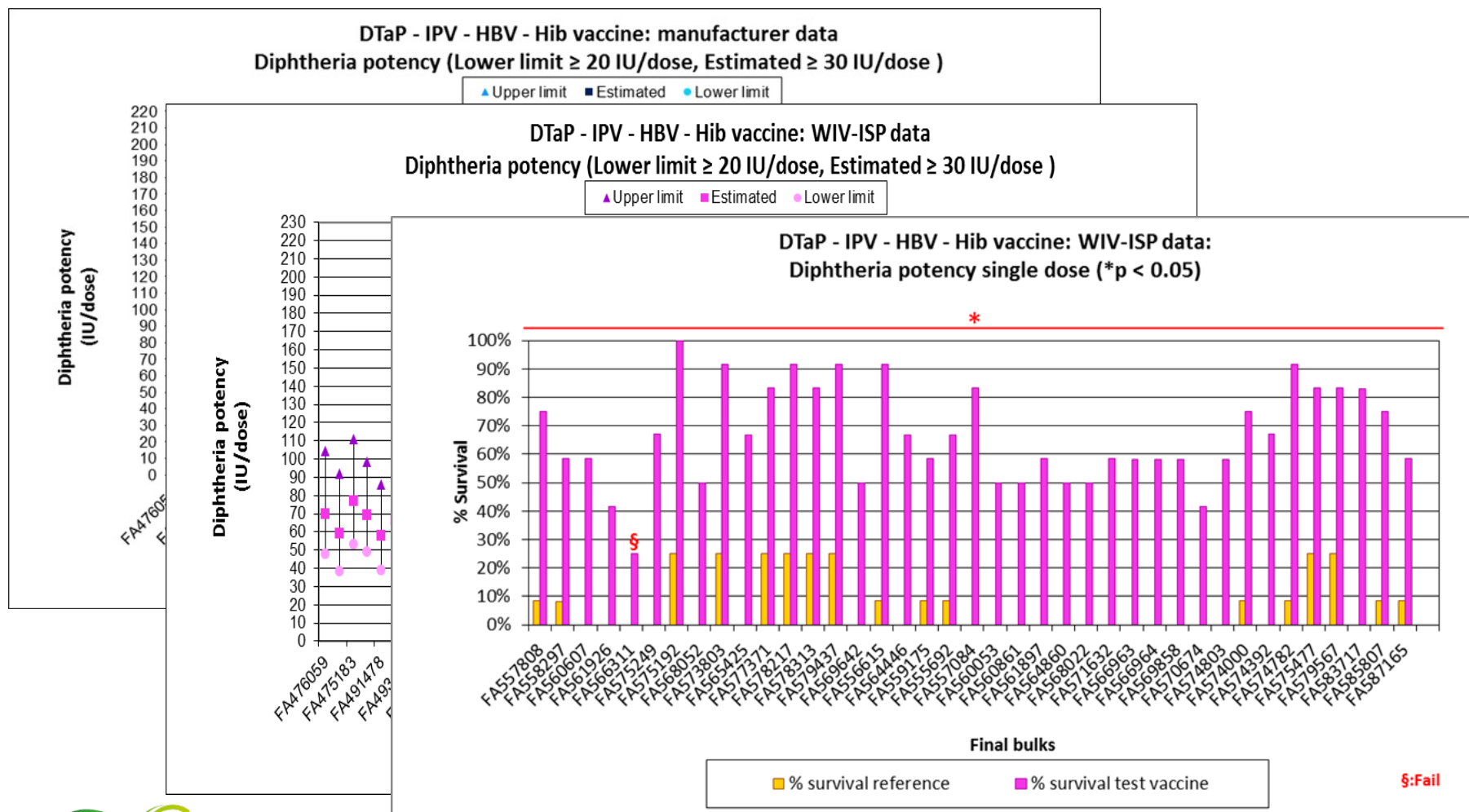
Veerle Van Melle
Anémone Gelfged



BACK-UP SLIDES

Additional info → Reduction Scheme

Example for data collected for Diphtheria potency



Additional info → Reduction Scheme

Example for data collected for Tetanus potency

DTaP - IPV - HBV - Hib vaccine: manufacturer data

Tetanus potency (Lower limit ≥ 40 IU/dose)

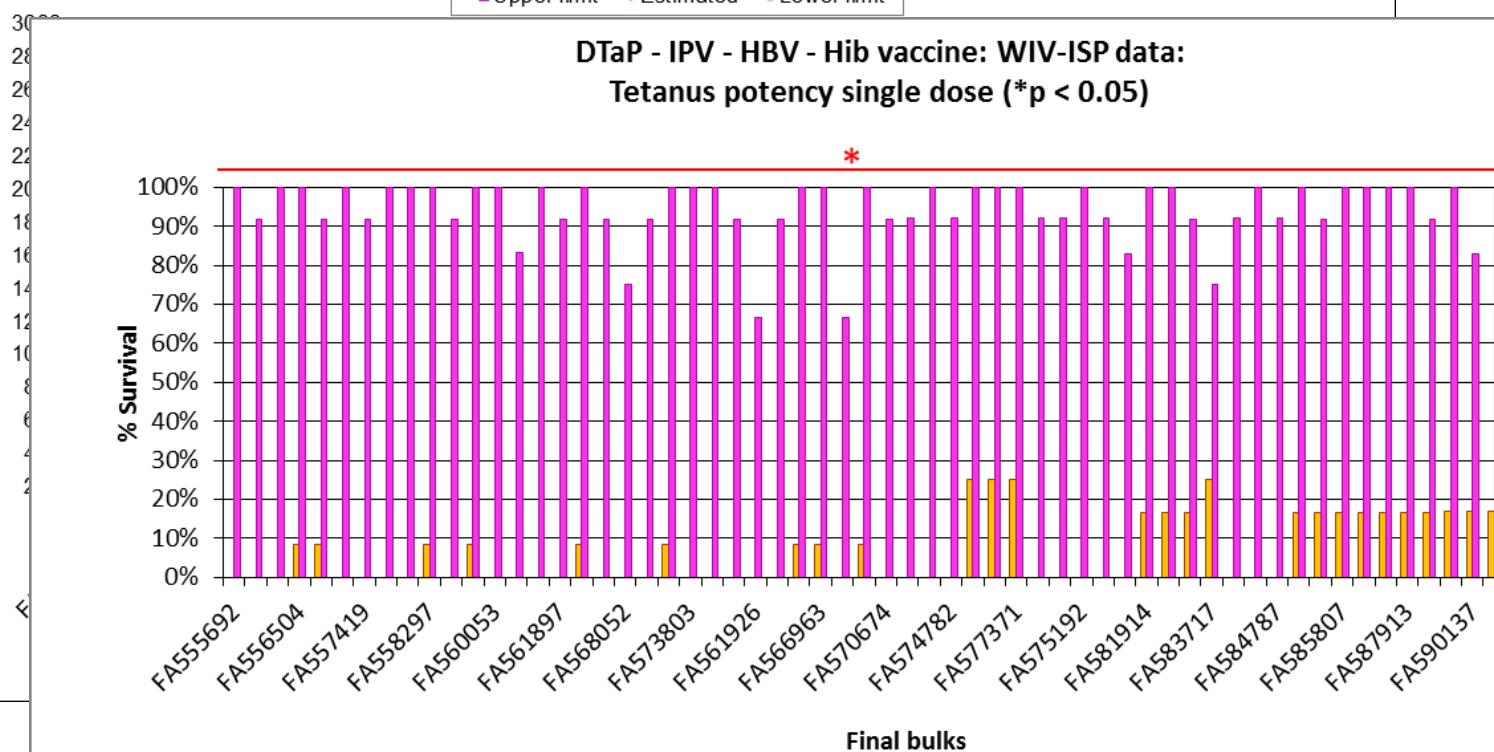
DTaP - IPV - HBV - Hib vaccine: WIV-ISP data

Tetanus potency (Lower limit ≥ 40 IU/dose)

▲ Upper limit ♦ Estimated ● Lower limit

Tetanus potency
(IU/dose)

Tetanus potency
(IU/dose)



% survival reference

% survival test vaccine