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Connects health, science and society

A MULTIPLEX IMMUNOASSAY FOR DTAP IN VITRO POTENCY TESTING

Global workshop.

Transitioning DTwP containing vaccines to animal free batch release testing strategy. Strategies for implementation.

Quality of vaccines and blood products (QVBP) – D&R
Maxime Vermeulen, PhD

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VAC2VAC

FUNDERS
IMI2 and EFPIA

TIMELINE
March 2016 to
February 2022

CONSORTIUM
23 partners
(DE, NL, UK, IT, BE,
FR, AT)

COORDINATOR
European Vaccine
Initiative

PRODUCTS:
7 Vaccine Franchises
5 veterinary, 2 human and 1 adjuvant

33 TASKS:
organized in **4 technical work packages** (WP)
to replace animal assays in Quality Control

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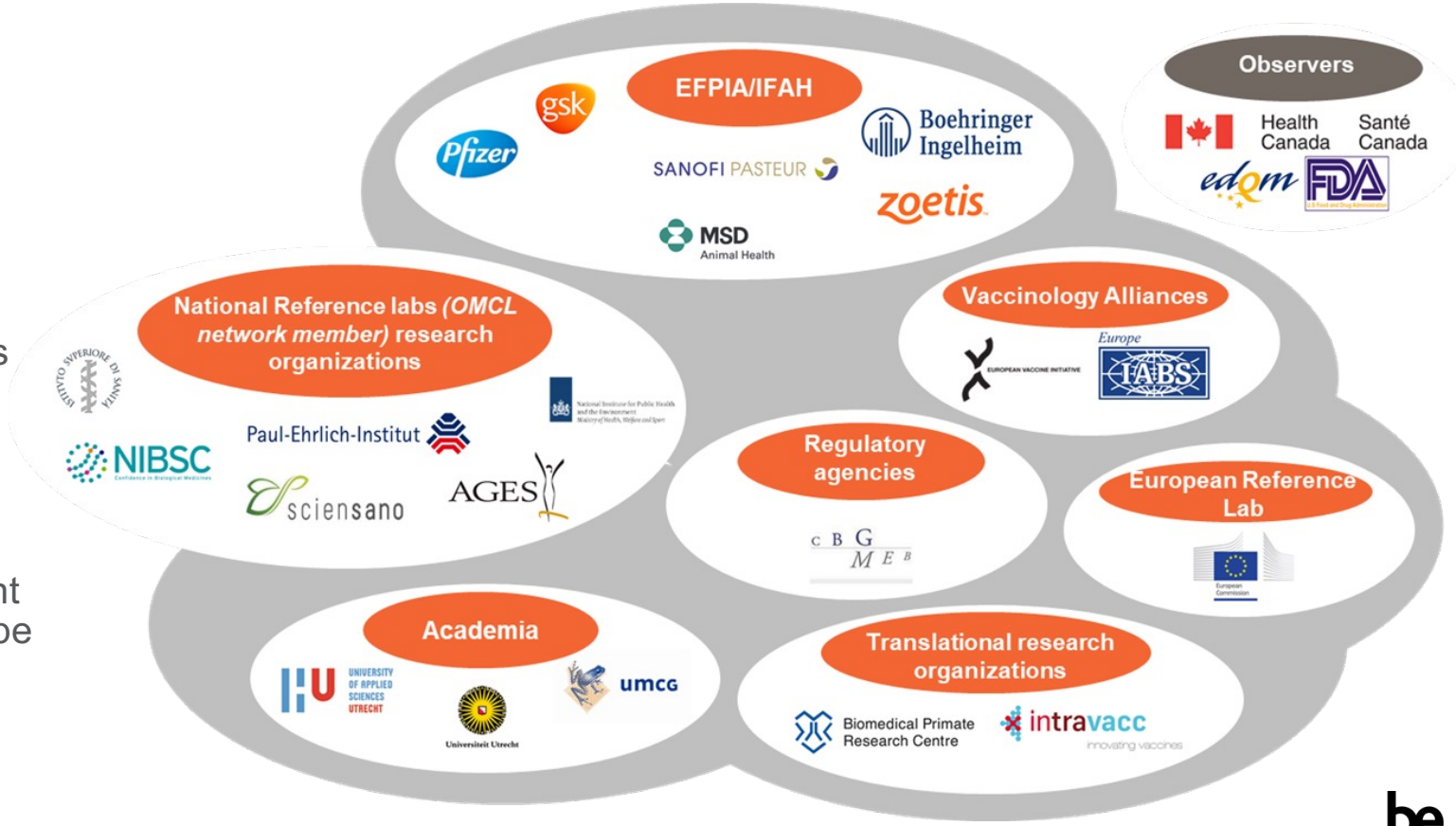
Affiliations of VAC2VAC Partners

7 types of affiliations

Working together to substitute animal assays for lifecycle vaccines

Observers ensure alignment within and outside Europe



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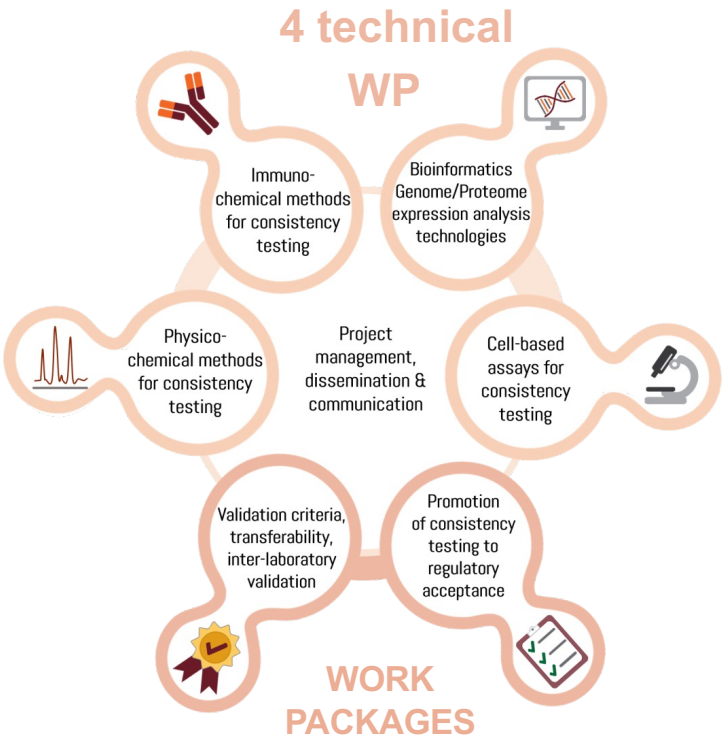
VAC2VAC - Objectives

The overall objective of the VAC2VAC project is to 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



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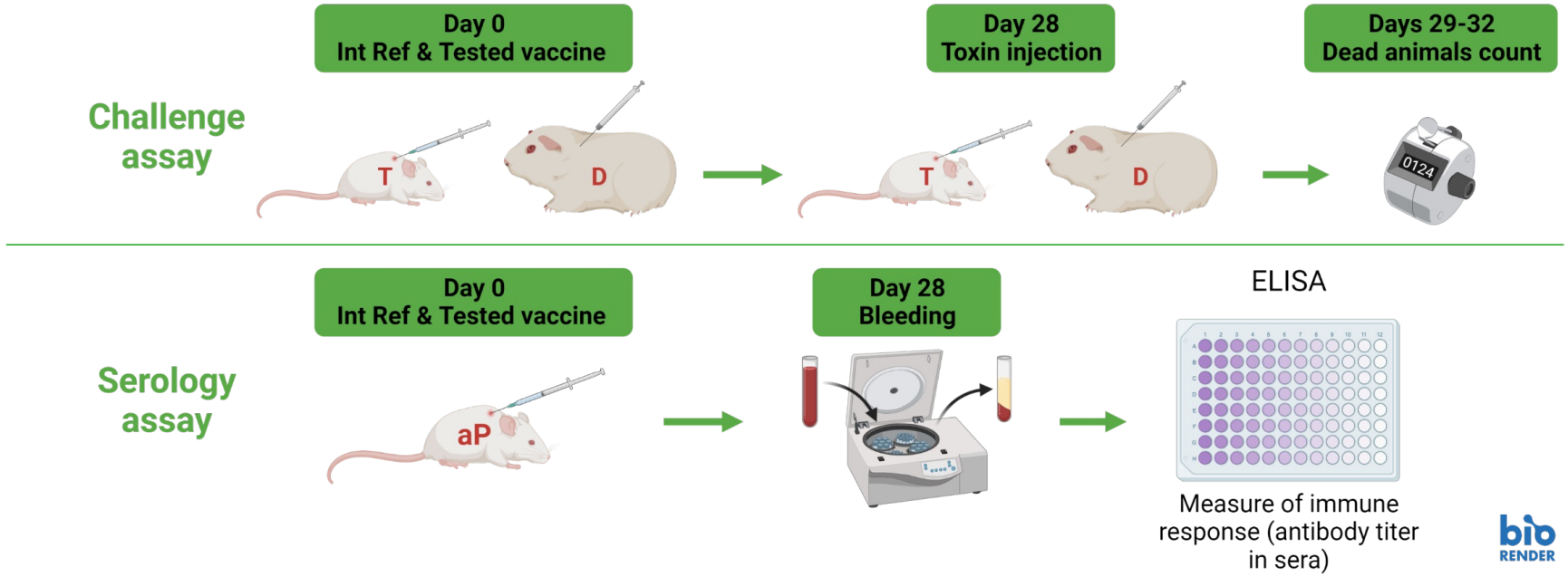
Animal use in the frame of DTaP vaccine quality control



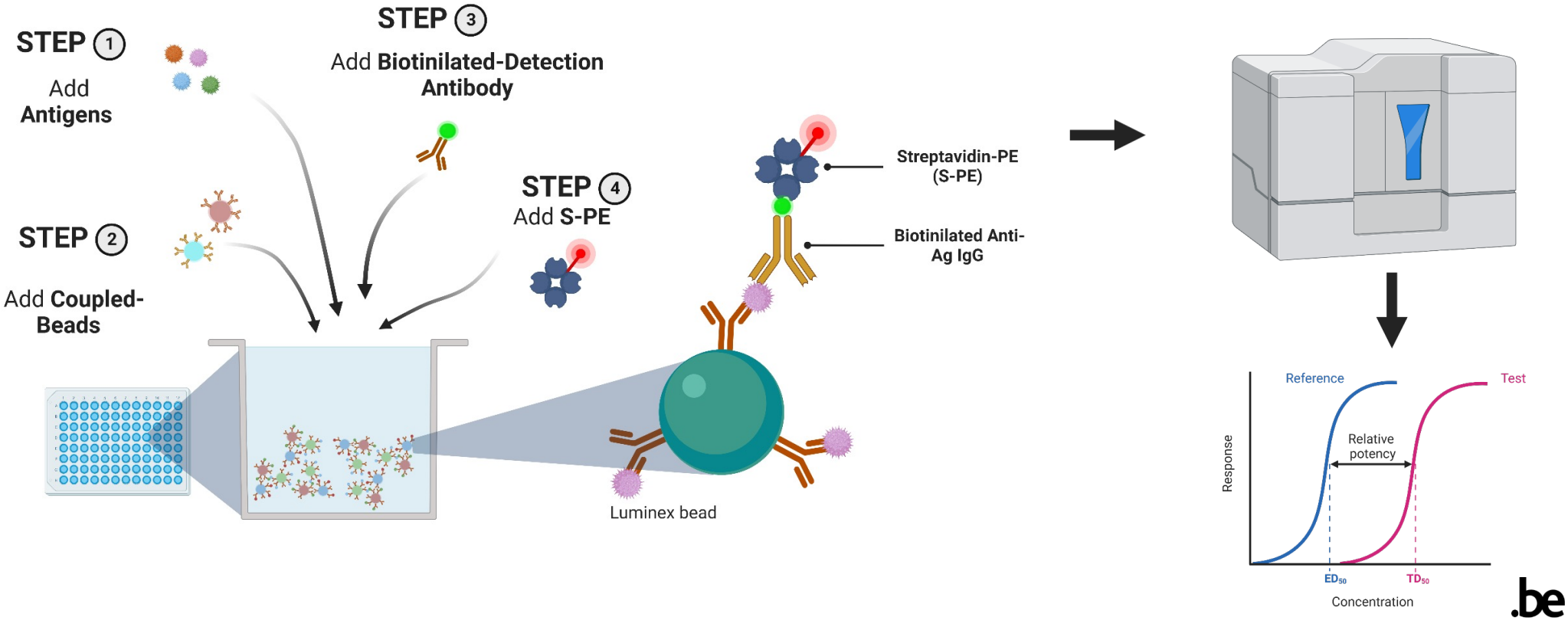
Ethical concerns
High cost
Prolonged testing period
High variability

→ Immunoassays to measure antigens' potency directly in vaccine

→ MHRA: D and T ELISAs

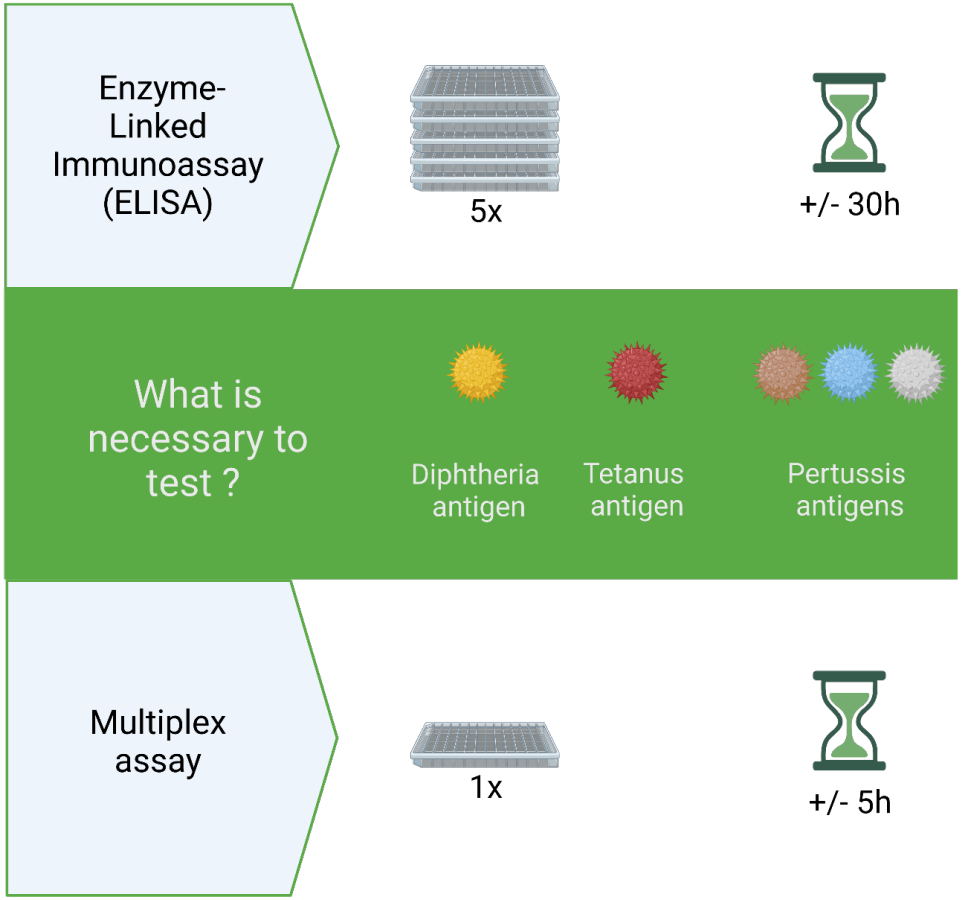
→ Sciensano: D, T and aP Multiplex

Mutliplex - Principle of the method



Multiplex immunoassay development for DTaP vaccines

- Multiplex advantage
  - ❖ One assay to assess 5 antigens
  - ❖ Faster (*vs.* std immunoassay *e.g. ELISA*)
- Multiplex inconvenient
  - ❖ More difficult to transfer
  - ❖ Market dependent (beads, device)
- Well characterized antibodies
  - ❖ 5 mAb pairs characterized and selected during VAC2VAC for DTaP antigens
- 8 vaccines from 2 human manufacturers (HuA – HuB)



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METHOD DEVELOPMENT

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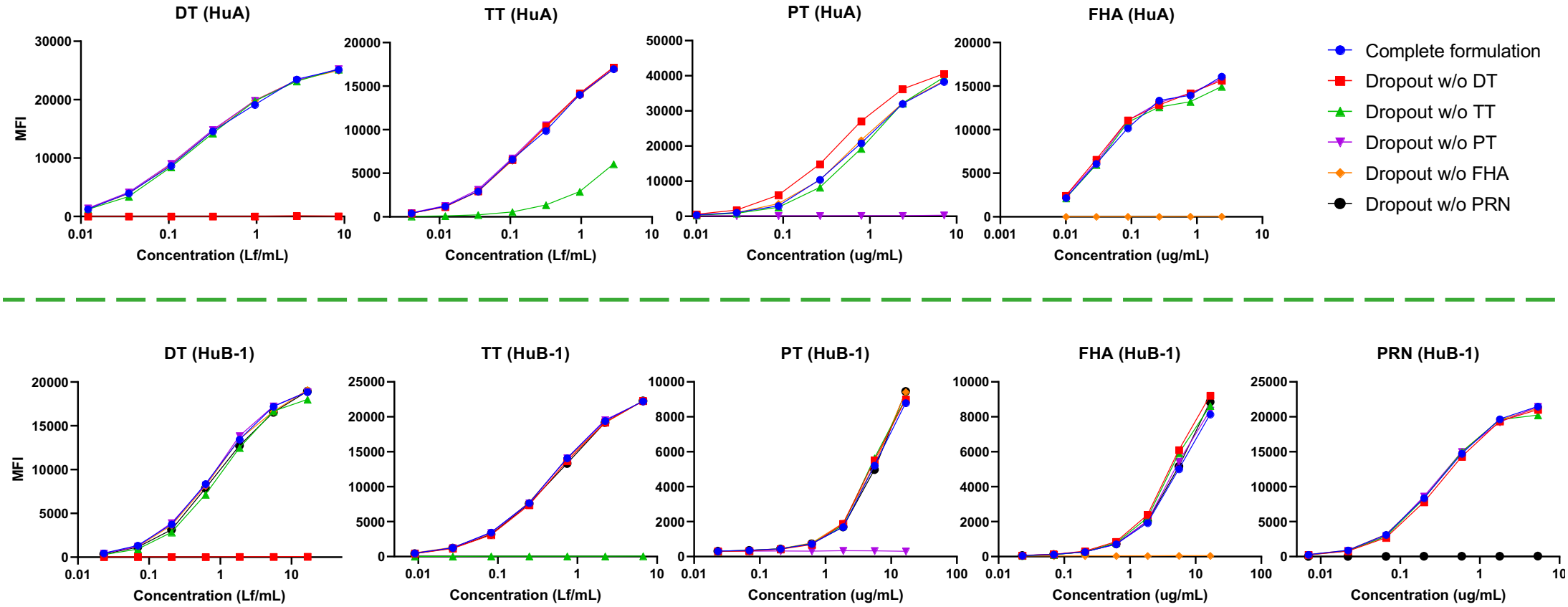
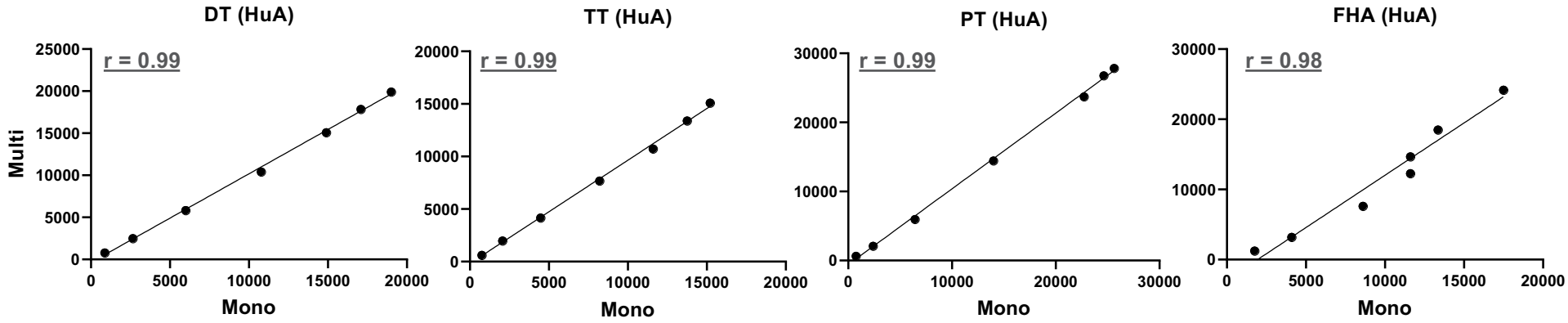
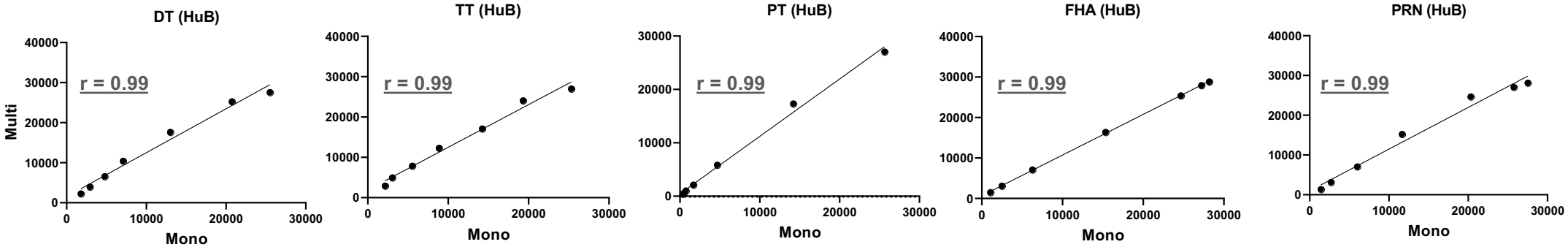
Assay specificity (drop-out samples)

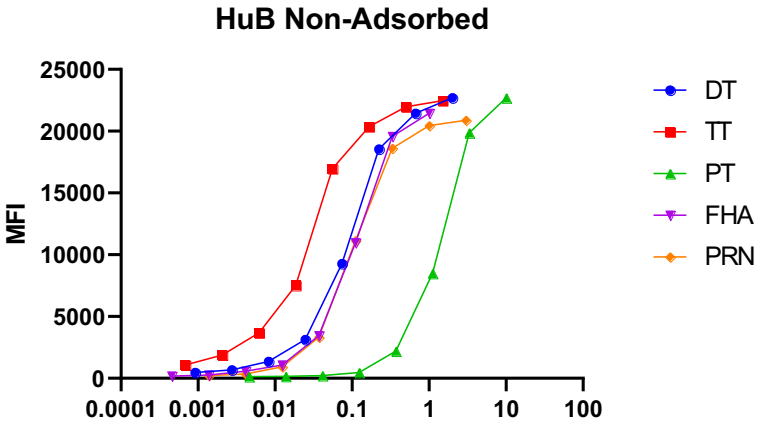


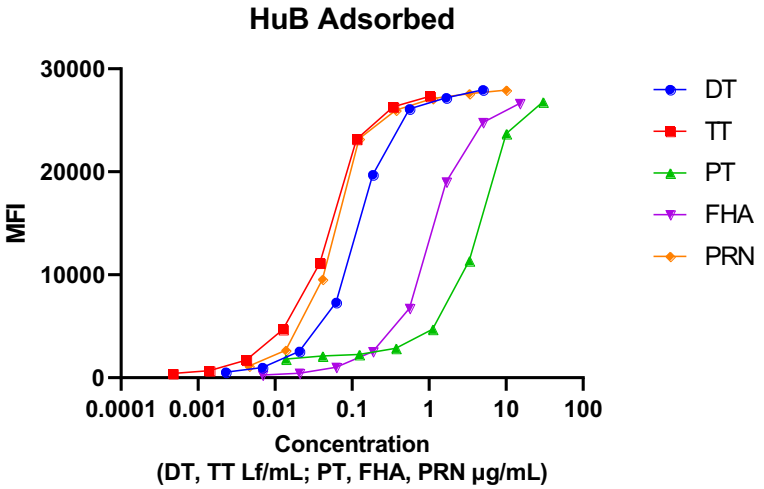
Cross-reactivity

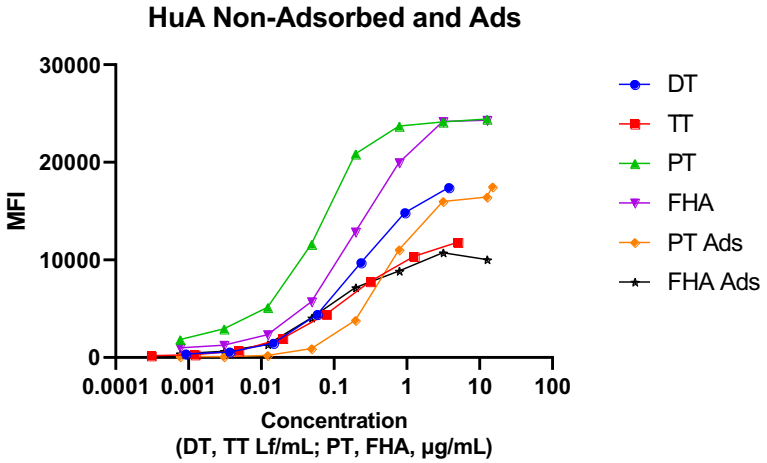




HuA and HuB antigens

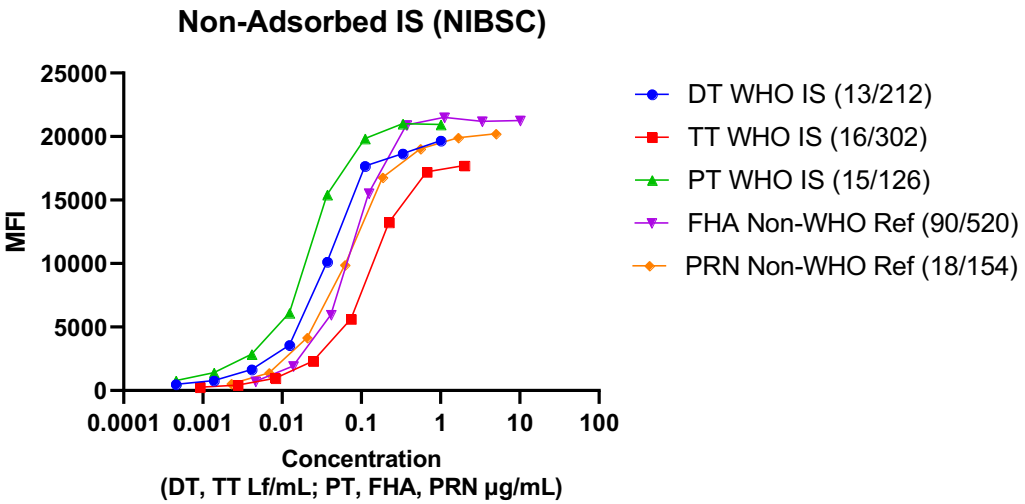


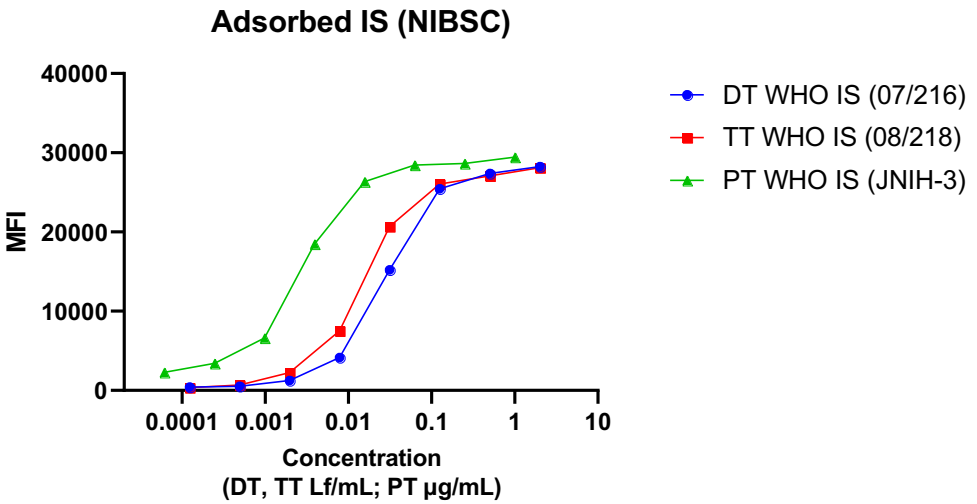




Dose response curves with non-adsorbed and adsorbed antigens

International standard antigens





Dose response curves with non-adsorbed and adsorbed international standards

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METHOD APPLICATIONS

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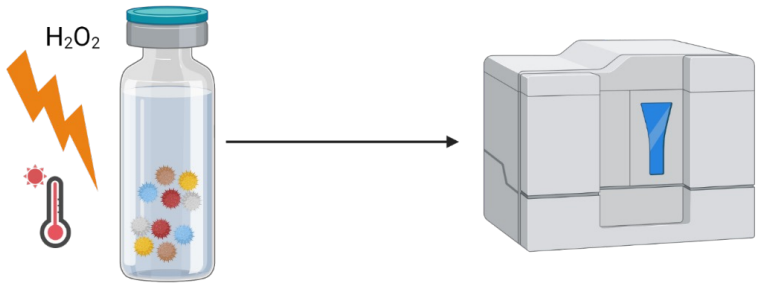
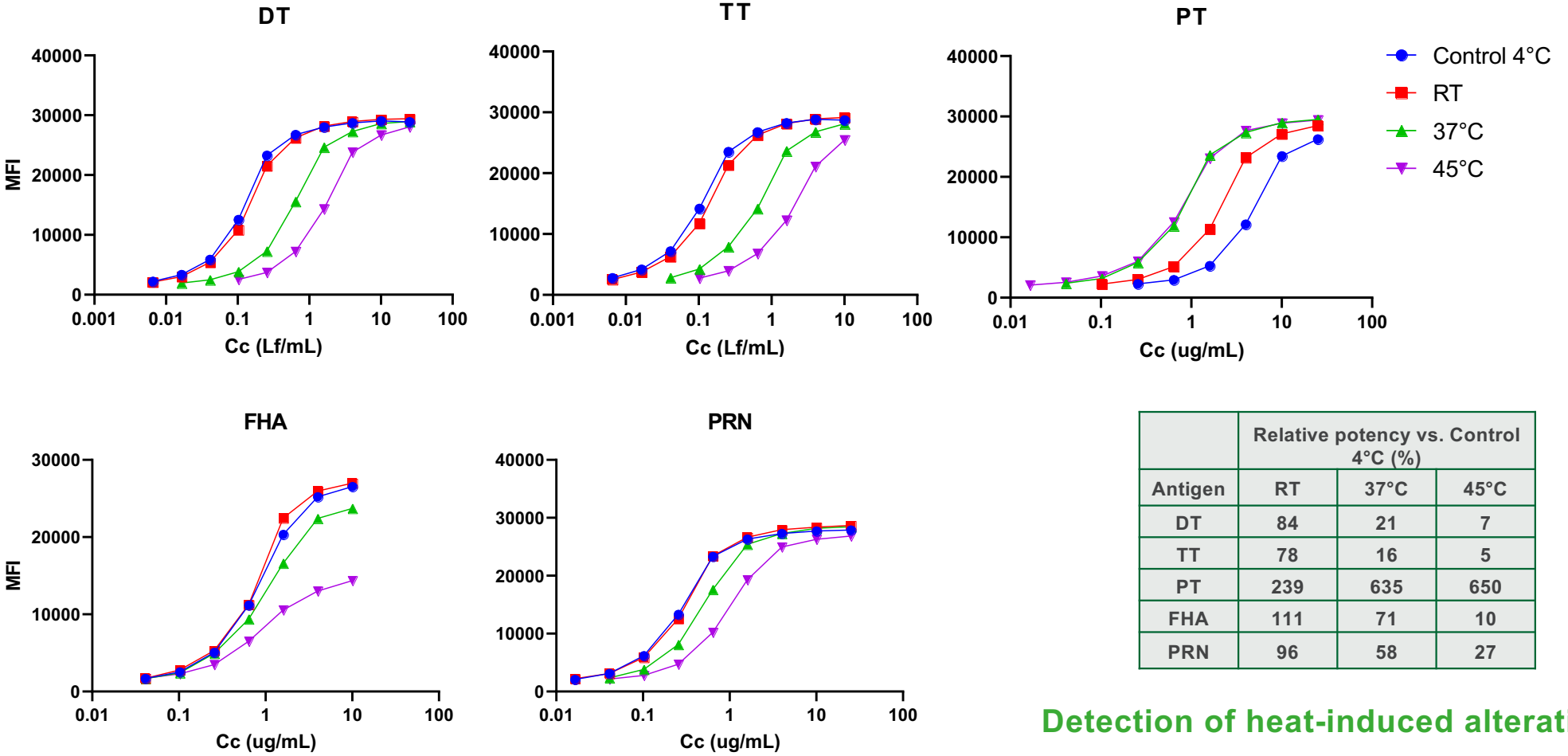
What the multiplex assay should detect ?

Ph.Eur. General Chapter 5.2.14: Substitution of In Vivo Methods by In Vitro Methods for the Quality Control of Vaccines:

1. Antigen functionality



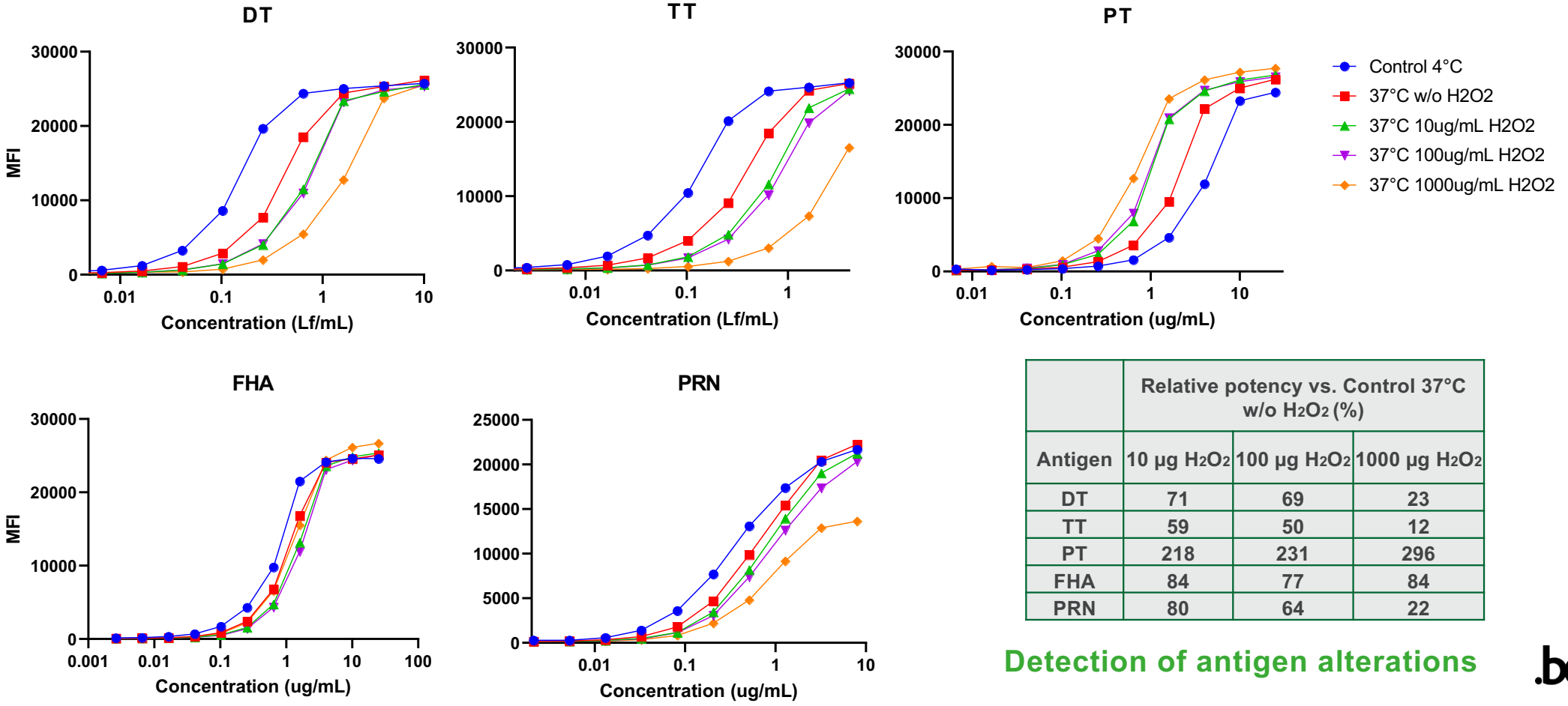
Detection of heat-induced alteration HuB DTaP vaccine



	Relative potency vs. Control 4°C (%)		
Antigen	RT	37°C	45°C
DT	84	21	7
TT	78	16	5
PT	239	635	650
FHA	111	71	10
PRN	96	58	27

Detection of heat-induced alterations

Detection of oxidation-induced alteration – HuB DTaP vaccine



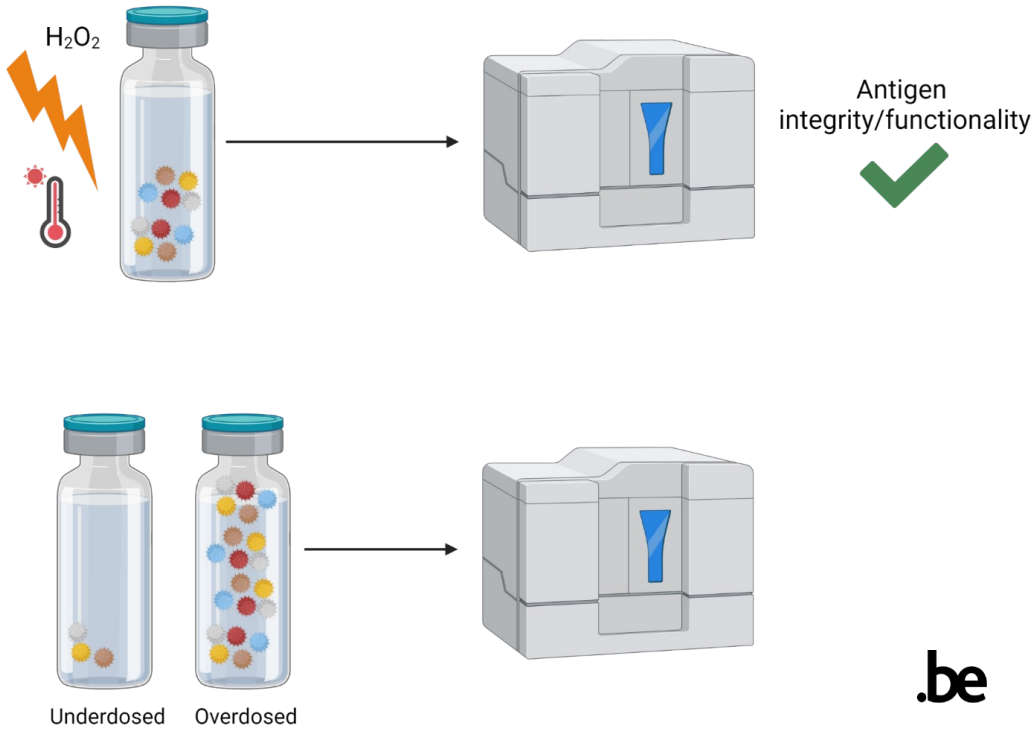
	Relative potency vs. Control 37°C w/o H_2O_2 (%)		
Antigen	10 µg H_2O_2	100 µg H_2O_2	1000 µg H_2O_2
DT	71	69	23
TT	59	50	12
PT	218	231	296
FHA	84	77	84
PRN	80	64	22

Detection of antigen alterations

What the multiplex assay should detect ?

Ph.Eur. General Chapter 5.2.14: Substitution of In Vivo Methods by In Vitro Methods for the Quality Control of Vaccines:

1. Antigen functionality
2. Antigen quantity



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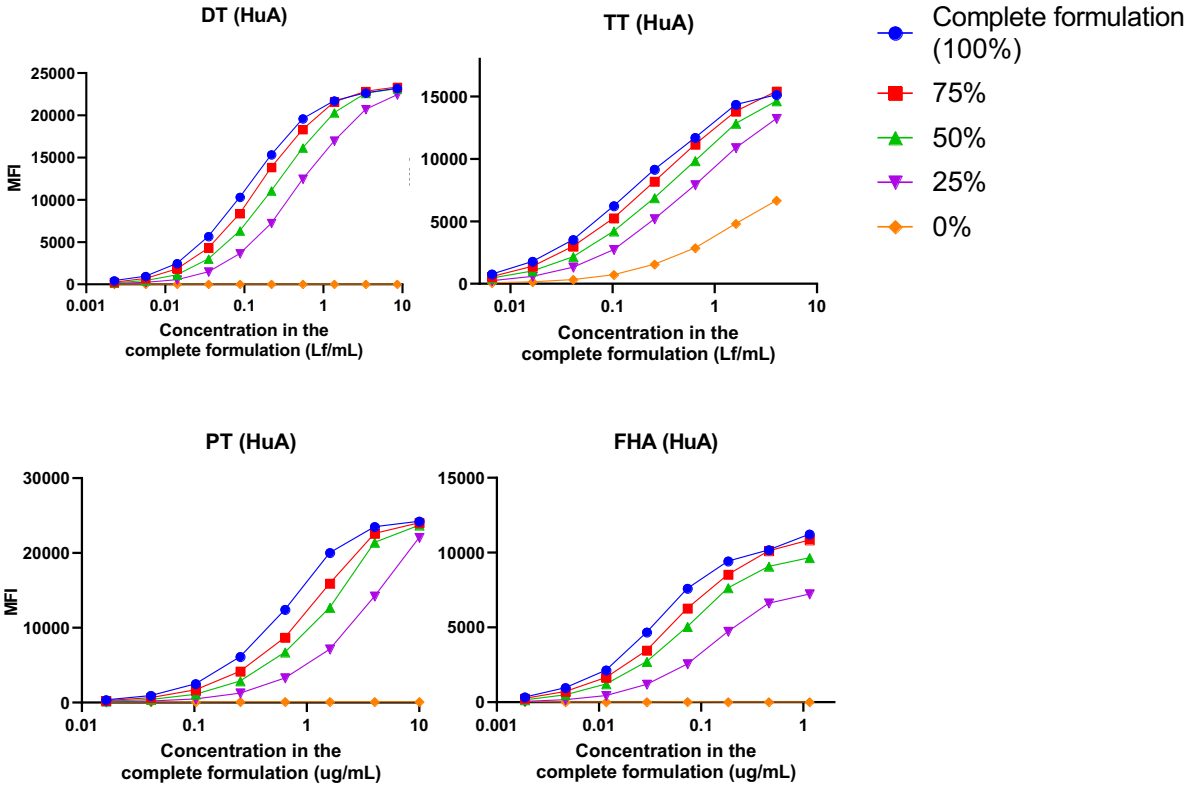
Detection of subpotent formulation - HuA



Antigens	Theoretical Ag relative amount (%)	Calculated Ag relative amount (%)	Recovery (%)
DT	75	73.8	98.4
	50	47.3	94.6
	25	23	92
TT	75	78.0	104.0
	50	51.9	103.8
	25	27.8	111.2
PT	75	61.9	82.5
	50	44.3	88.6
	25	20.2	80.8
FHA	75	66.3	88.4
	50	43.0	86.0
	25	12.2	48.8

Acceptable dilutional linearity (recovery 80-120%)

Detection of abnormally low amount of Ag in the formulation

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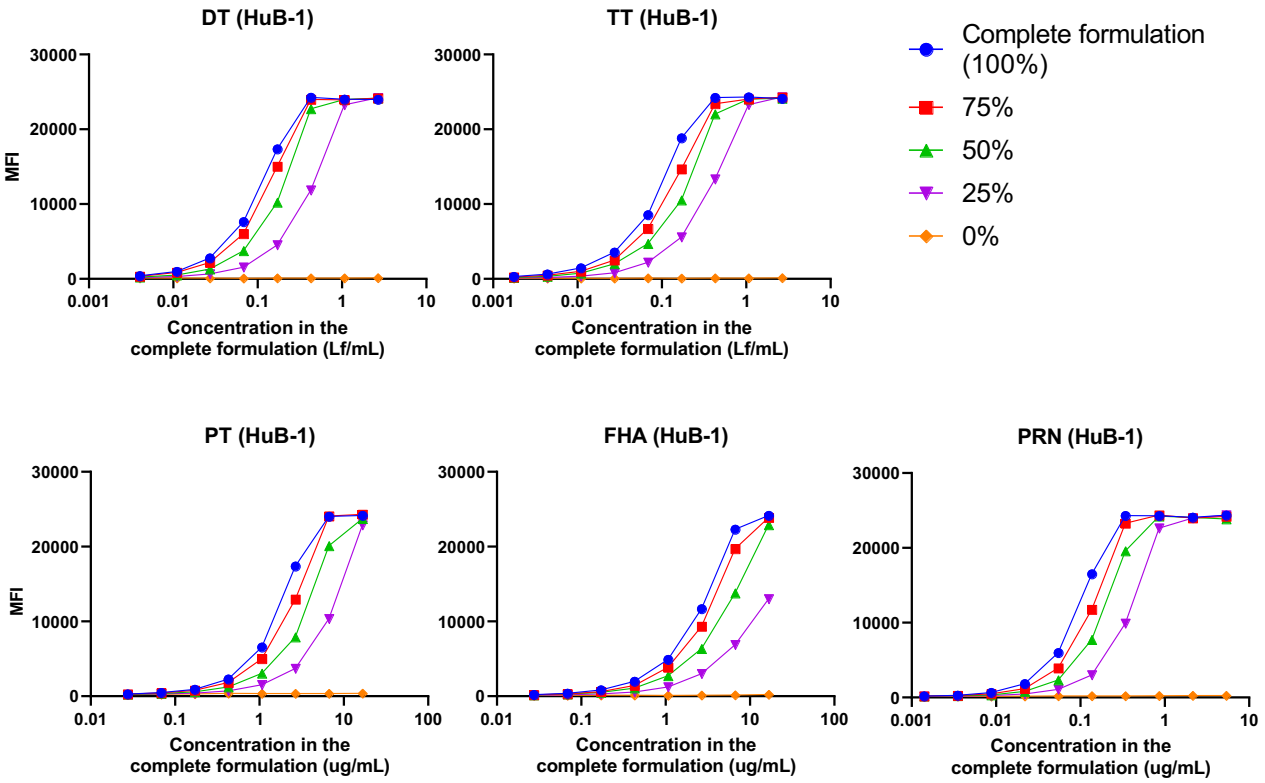
Detection of subpotent formulation - HuB



Antigens	Theoretical Ag relative amount (%)	Calculated Ag relative amount (%)	Recovery (%)
DT	75	81.8	109.1
	50	56.9	113.8
	25	25.8	103.2
TT	75	72.2	96.3
	50	51.4	102.8
	25	25.7	102.8
PT	75	74.8	99.8
	50	48.4	96.8
	25	24.5	98
FHA	75	77.7	103.6
	50	48.8	97.6
	25	18.2	72.8
PRN	75	70.9	94.5
	50	50	100
	25	24.9	99.6

Acceptable dilutional linearity (recovery 80-120%)

Detection of abnormally low amount of Ag in the formulation

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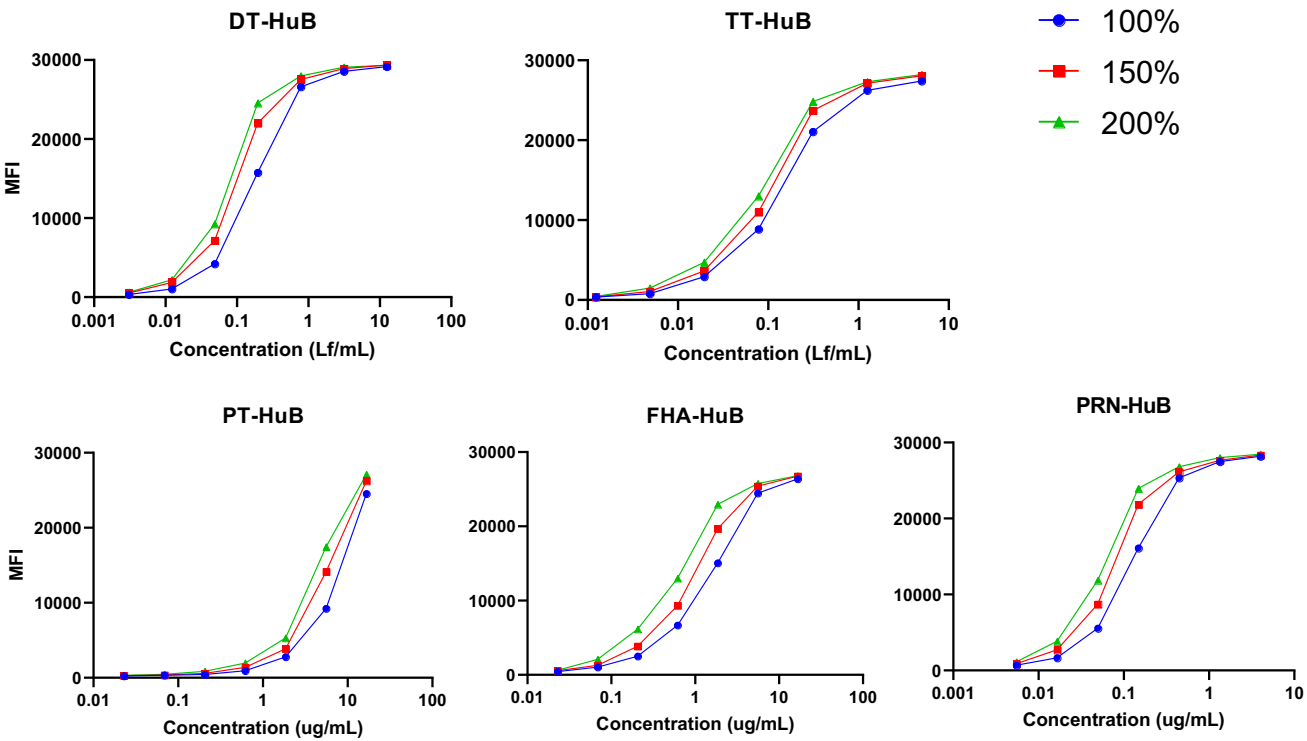
Detection of suspotent formulation - HuB



Antigens	Theoretical Ag relative amount (%)	Calculated Ag relative amount (%)	Recovery (%)
DT	150	176	118
	200	230	115
TT	150	135	90
	200	171	85
PT	150	138	92
	200	177	89
FHA	150	156	104
	200	247	124
PRN	150	161	107
	200	213	106

Acceptable dilutional linearity (recovery 80-120%)

Detection of abnormally high amount of Ag in the formulation

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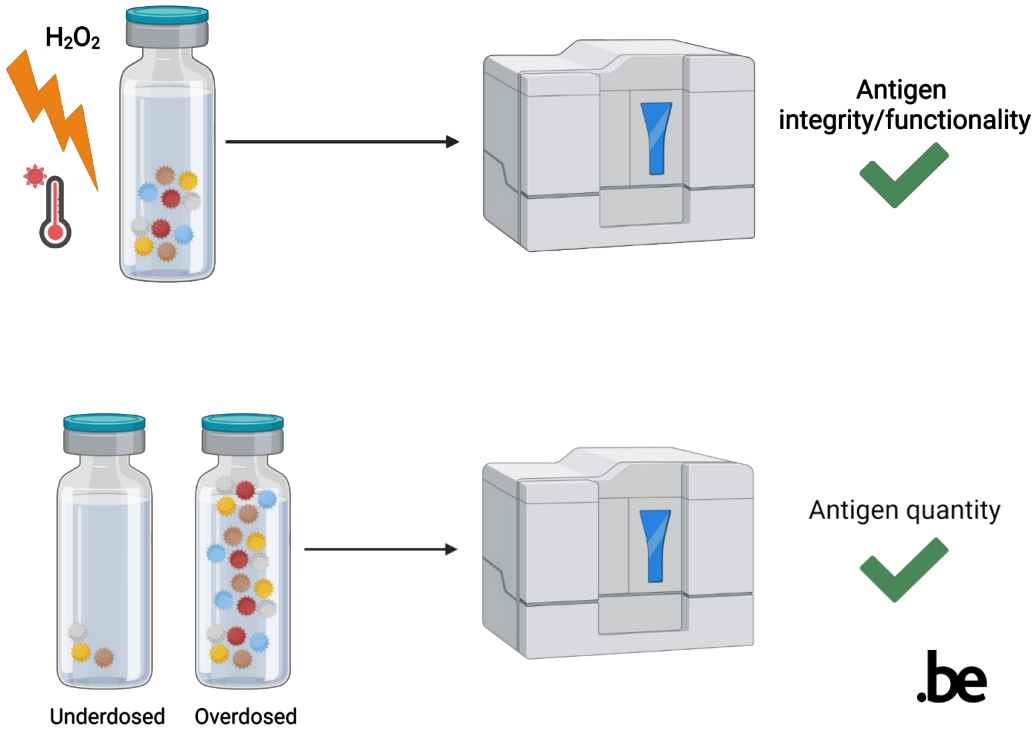
What the multiplex assay should detect ?

Ph.Eur. General Chapter 5.2.14: Substitution of In Vivo Methods by In Vitro Methods for the Quality Control of Vaccines:

1. Antigen functionality
2. Antigen quantity
3. Production consistency monitoring



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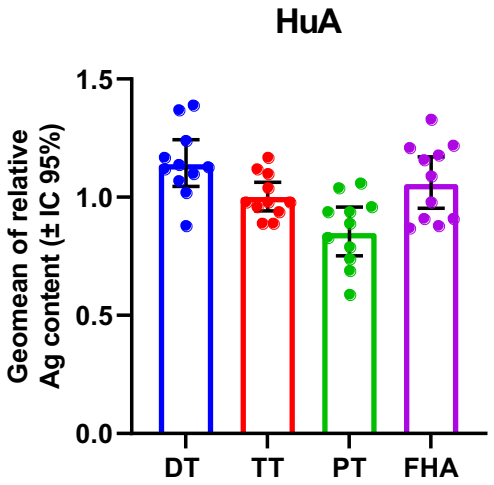
Batch to batch consistency - HuA

Calculation of relative potency using the CombiStats™ software with one vaccine batch fixed as reference



n=12		95% Confidence Interval			
Antigen	**RP***	LL	UL	LL in %	UL in %
DT	**1.14**	1.05	1.24	92%	109%
TT	**1.00**	0.94	1.06	94%	106%
PT	**0.85**	0.75	0.96	89%	113%
FHA	**1.06**	0.95	1.17	90%	111%

**The relative potency (RP) of one sample compared with another is defined as the ratio of equally effective doses for the samples (Finney 1964), and RP bioassays are designed to measure the potency of a test batch of material relative to a reference standard.*

→ Good consistency

→ Low batch to batch variability

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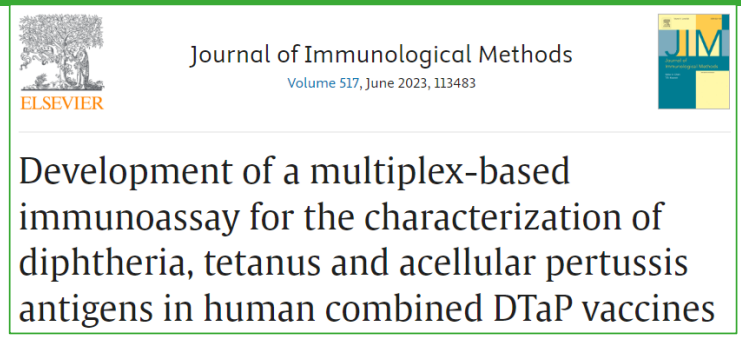
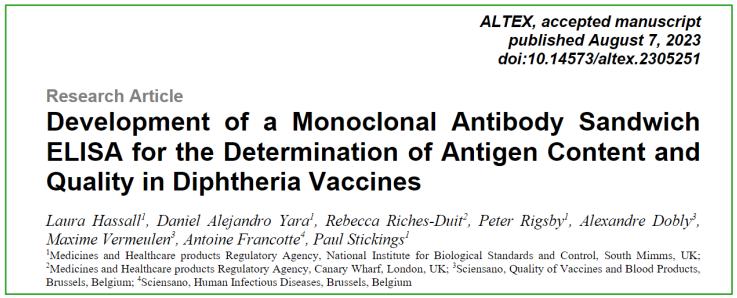
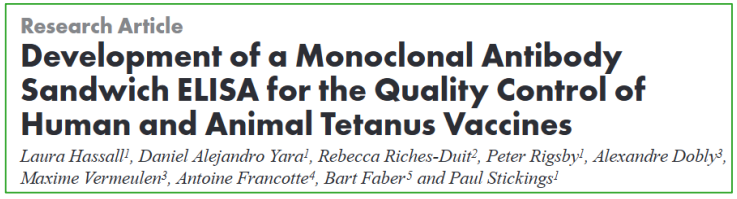
Where are we and where are we going ?

- Great enthusiasm from members of the consortium
  - Regulatory agencies
  - Pharma companies
- Methods transferred to manufacturers
- VAC2VAC-selected antibodies available on NIBSC website
- The ball is now in the court of the manufacturers
  - Discussion with regulators
  - Accumulation of data with *in vitro* potency assay
  - Submission of variation dossier to EMA







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Thanks for your attention

Questions ?

https://europevaccine.wixsite.com/vac2vac-eu

Back-up slides

Sciensano – Official Medicines Control Lab (OMCL)

Core activities:

- **Batch Release** of Biological Medicinal Products (human and veterinary vaccines & plasma-derived medicinal products)
- **Advising** during licensing and GMP inspections for human and veterinary 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)

METHOD APPLICATIONS

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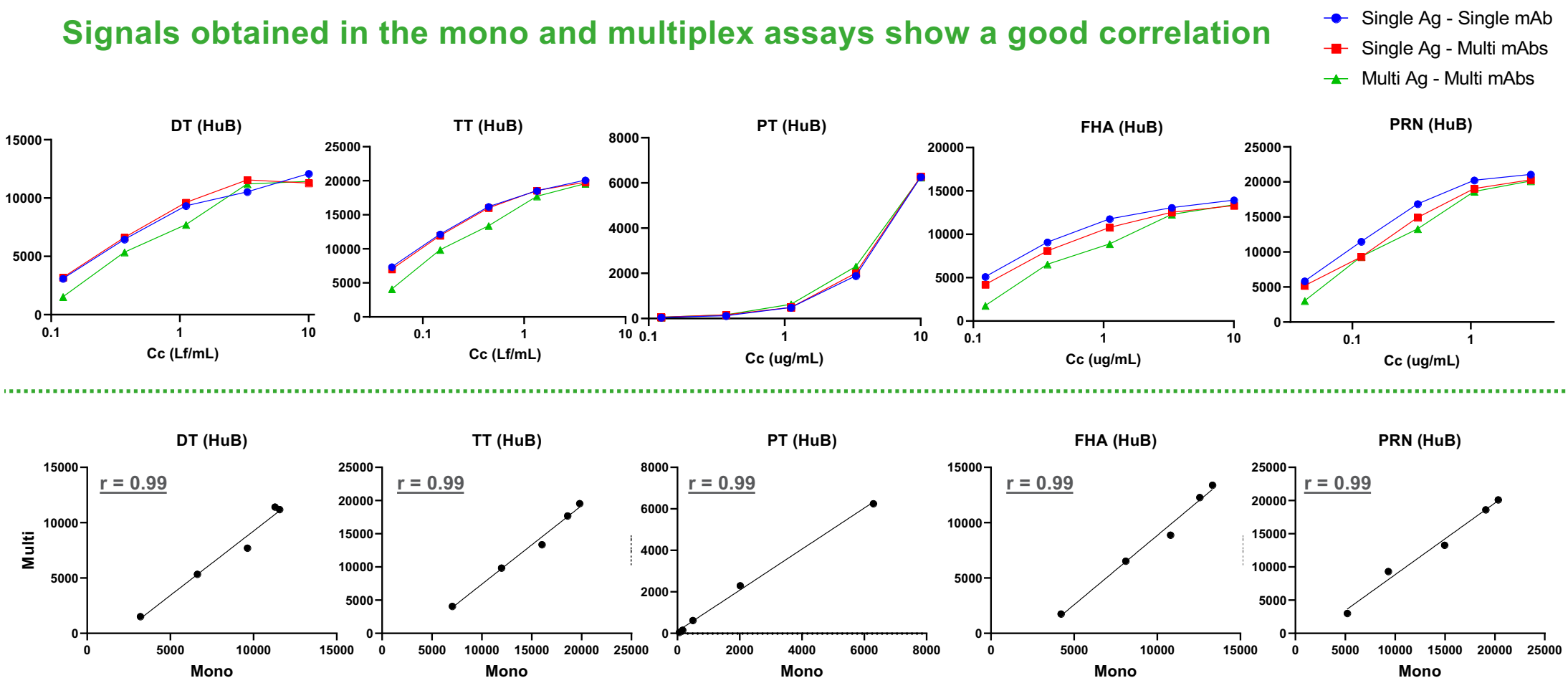
Cross-reactivity - HuB

Signals obtained in the mono and multiplex assays show a good correlation



Development of a multiplex immunoassay

- Multiplex → one run to assess all antigens
- Save animal, time and costs
- Lower variability than *in-vivo* methods (Stalpers *et al.*, Coefficients of Variance from 15% to 101%)

	Number of animals for *in-vivo* potency 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

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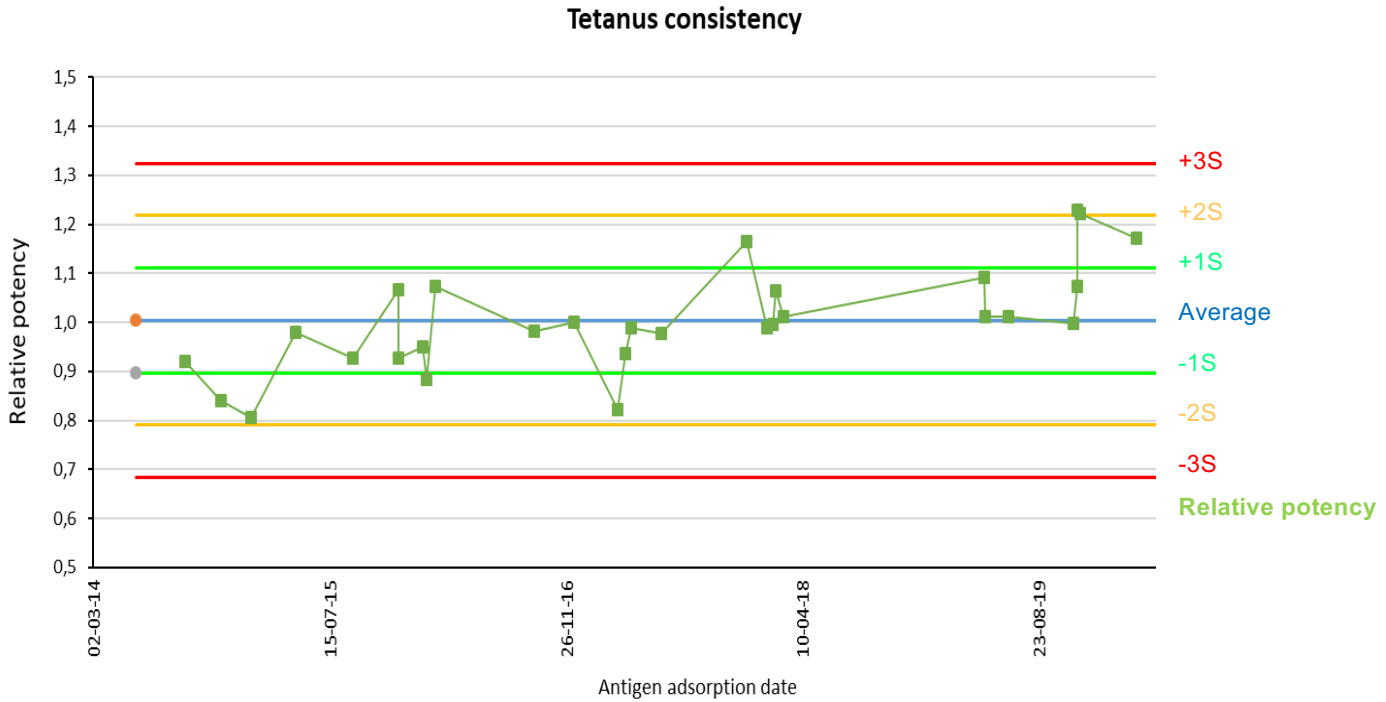
Luminex assay variability (12 plates)

Antigen	DTaP Vaccine (HuB)	GCV %
Diphtheria	High Dose	5%
	Low Dose	4%
Tetanus	High Dose	5%
	Low Dose	5%
Pertussis	High Dose	5%
	Low Dose	4%
Filamentous haemagglutinin	High Dose	8%
	Low Dose	8%
Pertactin	High Dose	5%
	Low Dose	5%

- Data from transfer study with one industry partner
- Relative potency calculated in CombiStats™ using one batch against a reference batch for two drug products
- Repeated on six plates in two different places

Flowchart example – Tetanus consistency



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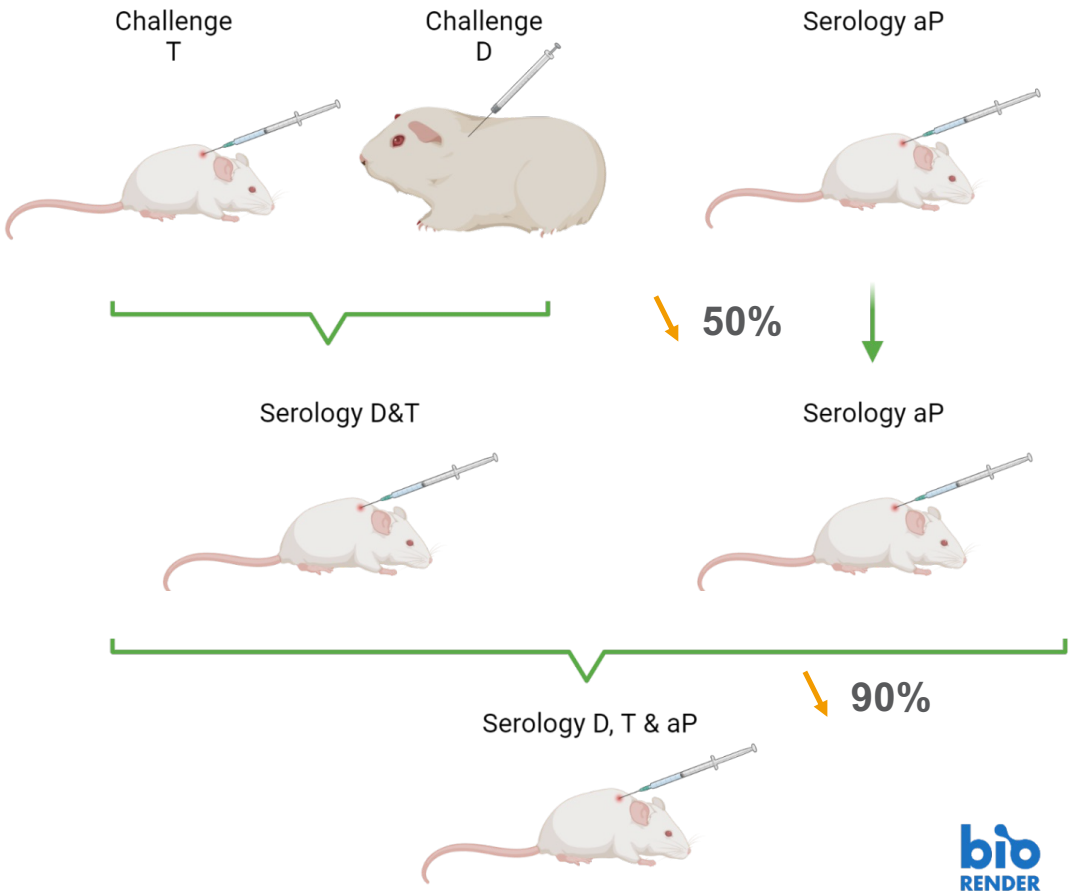
3R activities: reduce & replace

- Reduce
  - ❖ Substitution of multiple dilution Assay by single dilution Assay for D&T potency
  - ❖ Implementation of reduction scheme on DT vaccines

- Replacement
  - ❖ D&T challenge testing
    1. Serological testing (ongoing)
    2. *In vitro* (potency immunoassay)



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VACVAC accomplishments

Rabies in vitro potency assay

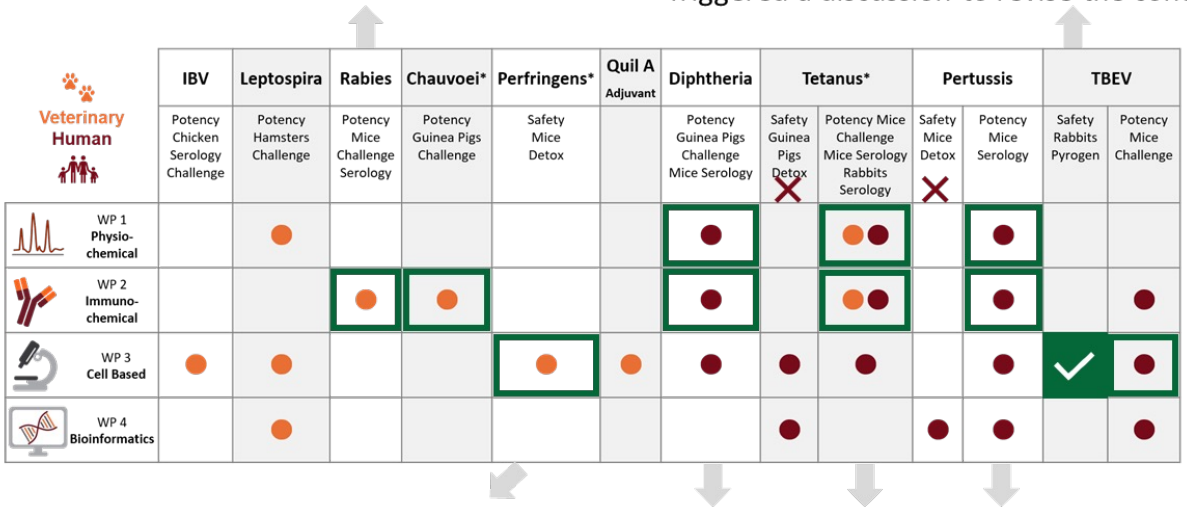
Strain-specific replacement ELISA have been designed. Validation ongoing / done / method filed (depending on manufacturer)

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



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 and transfer to industry partners achieved. Characterized antibodies commercially available (NIBSC website)

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Conclusion

- Many efforts are made to reduce/substitute animal testing in the frame of vaccine batch release
- Several *in vitro* assays developed during VAC2VAC
- Need some time to implement the assays in routine but a “mindset change” of regulators/manufacturers operated during VAC2VAC to move towards “animal-free” assays

Acknowledgement:

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Isabelle Feck
Alexandre Dobly

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