



Together, beyond animal health

In vitro potency test for companion animal rabies vaccines", in Brazil

AFSA LATAM WEBINAR, March 25, 2026

Regulatory and Scientific Perspectives: Case Studies in Replacing Animal Tests for Veterinary Vaccine Batch Release

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Topics discussed

- **Introduction of the vaccines**
Rabies vaccines for companion animals (e.g. dogs and cats)
- **ELISA as a potency test for the rabies vaccines**
Existing methods vs. in-house method development
- **Comparison of 3 ELISA methods**
Specificity and sensitivity
- **Optimisation and pre-validation of the in-house ELISA method**
Precision, LOD, LOQ, robustness
- **Next technical steps**

The rabies vaccine for companion animals

This rabies vaccine is targeted exclusively for the **Brazilian** market

Vaccine strain genotype 1; **PV strain** – also used in human rabies vaccines

Formulation For pets: - lyophilized – **non-adjuvanted** (as human vaccines)
- liquid – adjuvanted: Al(OH)₃ gel

In vitro methods to replace the NIH potency test

Start with the lyophilized vaccine for companion animals (no adjuvant)

– The virus strain and the formulation are similar to human vaccines

↳ The same method(s) may be used as for human rabies vaccines

Comparison of 3 strategies

- **Commercial kit:** Using a commercial rabies virus ELISA kit
Can be the fastest and easiest
- **Human ELISA:** Adapting the method developed by Sanofi-Pasteur for human vaccines*
The test was then validated by the **BSP148** collaborative project in the EU for human vaccines
- **In-house ELISA:** Developing an in-house ELISA method
Developed by March, 2022 and validated for the lyophilized pet vaccine by January, 2023

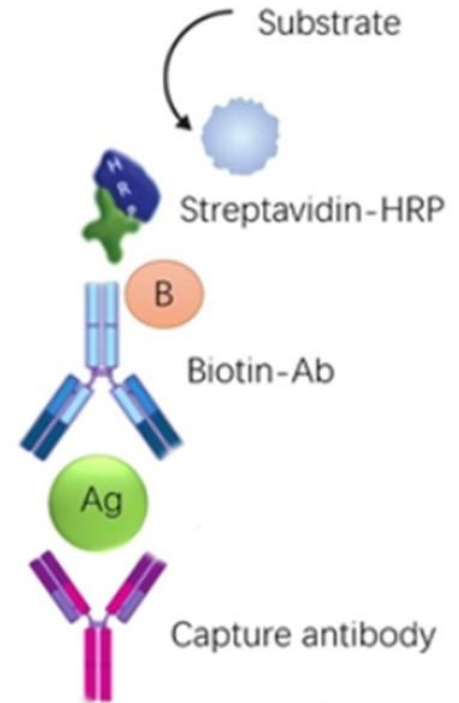
* Chabaud-Riou M, et al G-protein based ELISA as a potency test for rabies vaccines. *Biologicals*. 2017 Mar;46:124-129

Assay design

The setup of the assay is the same for all three methods

	Capture Ab (CAB)	Biotin conjugated detection Ab (DAB)
commercial kit	GP*-specific mAb	GP-specific mAb
human vaccine ELISA	Clone 1112 GP-specific, neutralizing mAb	Clone D1-25 GP-specific mAb
in-house ELISA	Clone 4F1 GP-specific mAb binding to a linear epitope	Clone 1C5, GP-specific, neutralizing mAb

* GP = glycoprotein



Method 1: commercial ELISA kit

Well-to-well **variability** of OD replicates is **high** --> justified with uniformity test

Day-to-day **intermediate precision** is **not acceptable**

Sensitivity for vaccine is **low**

Risk: dependence on an **external supplier**

Method 2: human vaccine ELISA

Chabaud-Riou M, et al G-protein based ELISA as a potency test for rabies vaccines. Biologicals. 2017 Mar;46:124-129

**Replacing the in vivo potency test for human rabies vaccine:
a global collaborative initiative**

BSP148 project

Project Leaders **Jean-Michel Chapsal (Independent/EPAA, FR)**

❖ 27 Feb. 2026 *BSP148 Technical debriefing meeting (Brussels, BE)*



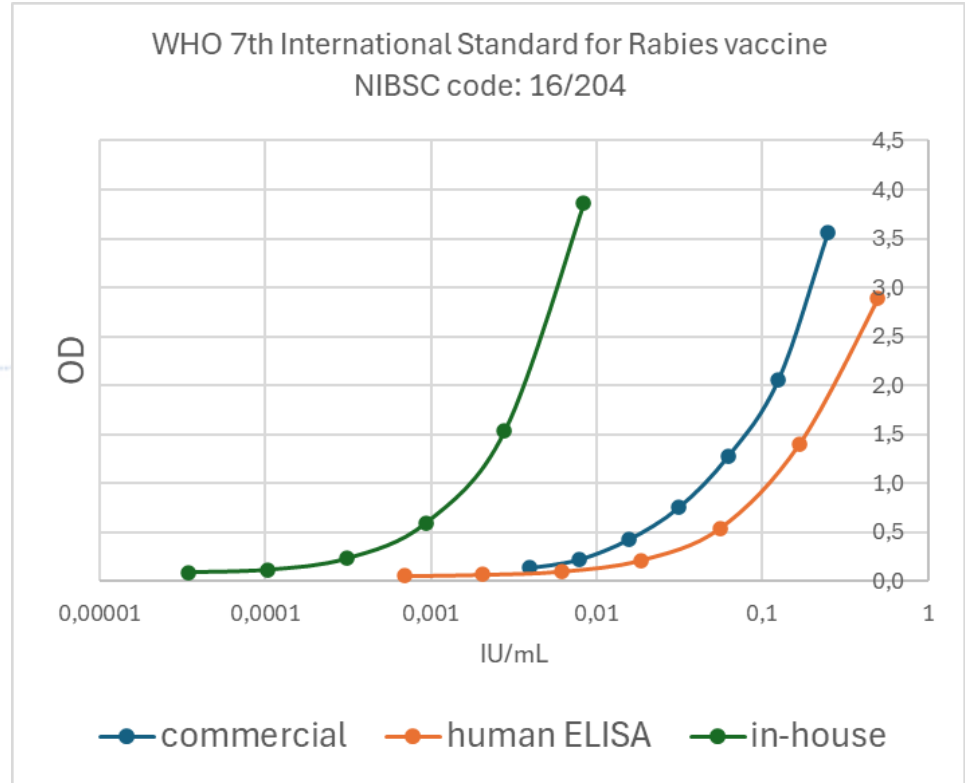
Comparison of the 3 ELISA methods

- **Specificity**

All methods use **GP-specific** monoclonal antibodies

- **Sensitivity**

The **in-house ELISA** is the most sensitive, about 20 times more sensitive than the **human vaccine ELISA**



Pre-validation of the in-house ELISA*

Standard WHO 7th International Standard for Rabies vaccine, NIBSC code: 16/204

Control Antigen bulk (PV strain) produced by Ceva Brazil

Test items 2-2 batches of antigen bulks and lyophilized pet rabies vaccine

	starting dilution	serial dilution
standard (WHO 7th IS)	1:400	1:2
internal control	1:1000	1:2
antigen sample	1:1000	1:2
vaccine sample	1:250	1:2

*Study No IMP-008-2023



Pre-validation of the in-house ELISA - precision

type	test method	measured maximum RSD%	RSD%
intra-assay	4 independent dilution series of a sample on the same plate, by 2 operators	4.8%	<5%
well-to-well	uniformity (same STD dilution in 96 wells, OD~1)	6.2%	No edge effect
inter-assay	10 independent test runs by 3 operators	9.2%	<10%

Pre-validation of the in-house ELISA - sensitivity

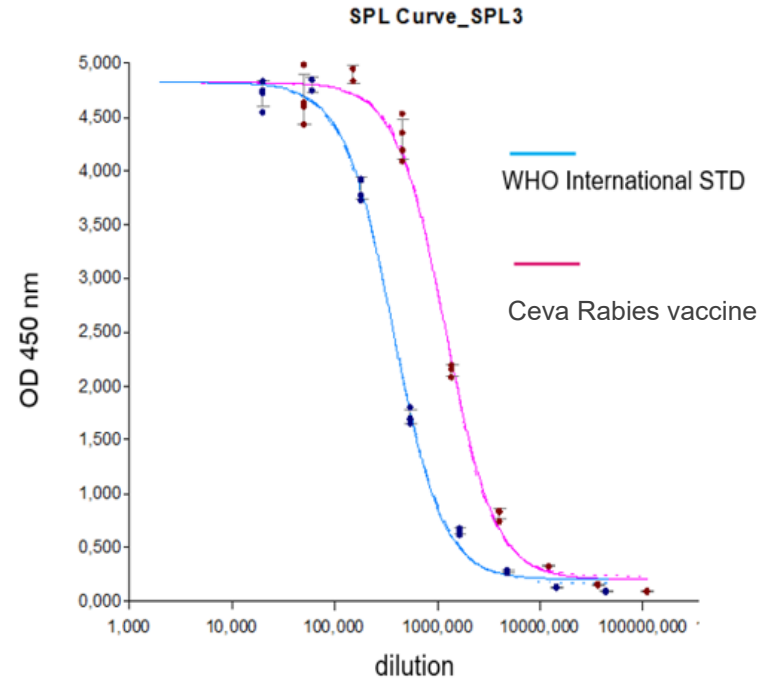
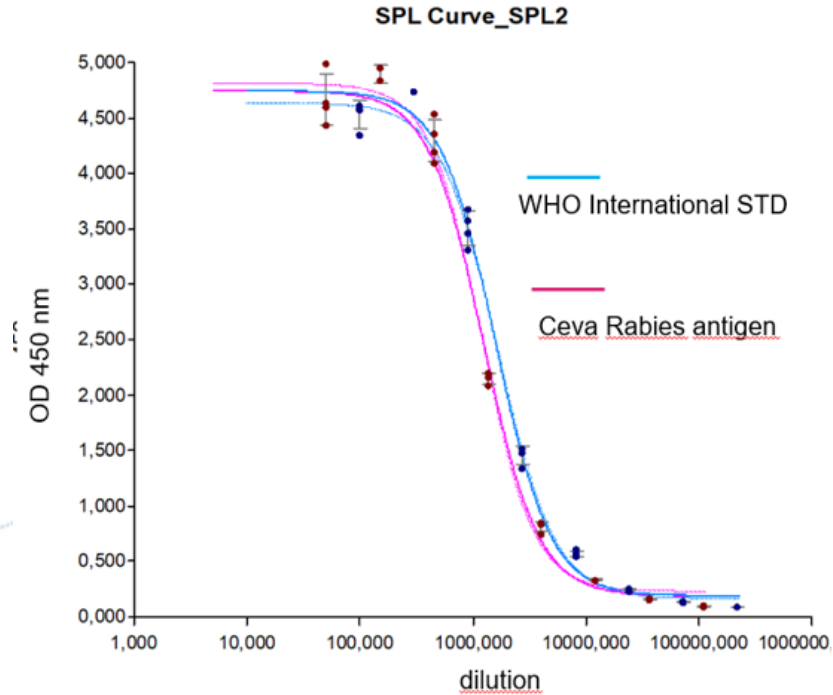
Detection limit (LOD) and quantification limit (LOQ) determined

LOD: VICH GL2 6.3.1 - Based on standard deviation of the blank

LOQ: VICH GL2 7.3.1

	concentration in the well	what does it mean?
LOD	0.000004 IU/mL	vaccine in 1 : 400.000 dilution can be detected
LOQ	0.00003 IU/mL	vaccine in 1 : 50.000 dilution can be quantified

Pre-validation of the in-house ELISA – dilution parallelism



Perfectly parallel lines

Pre-validation of the in-house ELISA – robustness

The aim was to develop a transferable method – the assay proved to be robust

type of parameters	investigated parameters	effect on the signal	effect on the results
raw materials /reagents	concentration of CAB \pm 20%	no	no
	concentration of biotin conjugated DAB \pm 20%	no	no
	lot of biotin conjugated DAB	no	no
	concentration of Streptavidin-HRP reagent \pm 20%	no	no
	Manufacturer of ELISA plate (Nunc, Greiner, Costar, Sarstedt)	not significant	no
	Type of TMB substrate (ready-to-use commercials)	not significant	no
incubation time	reaction time of sample reaction (\pm 20%)	no	no
temperature	37°C \pm 3 °C	no	no
	25°C (RT)	yes	no
shaking	shaking or without shaking	yes	yes*
washing	washing mode (automatic or manual)	no	no

**yes if the std is the WHO international standard but "no" if the std is an internal antigen*

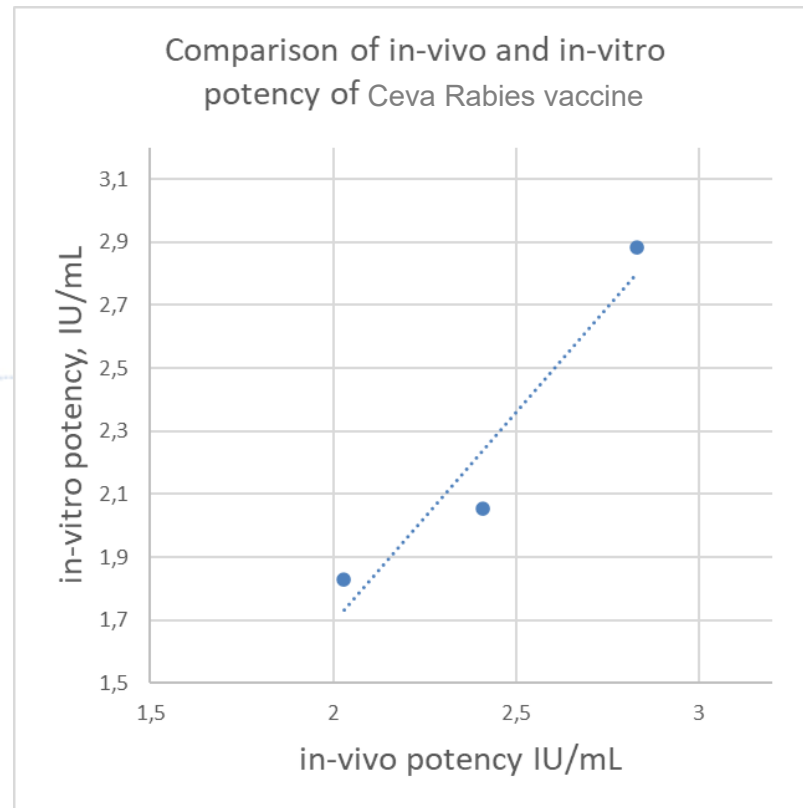


Comparison with NIH mouse potency

Vaccine Lyophilized Rabies vaccine	Potency in-vivo (NIH - mouse) IU/mL	Potency in-vitro in-house ELISA IU/mL
Batch #1	2.83	2.88
Batch #2	2.03	1.83
Batch #3	2.41	2.06

Good correlation

Limited number of data



Next technical steps

Batch potency test

Rabies vaccines, in general, are being evaluated and ELISA is being tested and validated for each type of formula.

In-process antigen quantification method

Replace the **CRO developed IPC antigen quantification ELISA** with the **in-house ELISA**



Thank you for your attention

