Section XII – Vaccines: Making difference on reduction.
Chair: Patrícia dos Santos Carneiro
(Instituto Butantan , SP).

INSTITUTO BUTANTAN

Alternative methods insertion into the QC lab routine: status and challenges

Patrícia dos Santos Carneiro, PhD
Manager of Quality Control and Analytical Development
Aug 24, 2018

2nd PAN-AMERICAN Conference for Alternative Methods
August 23-24, 2018
Rio de Janeiro

Butantan Institute: 116 years old of history

www.butantan.gov.br

- Since 1901
- Responsible for the majority of sera and vaccines used in Brazil

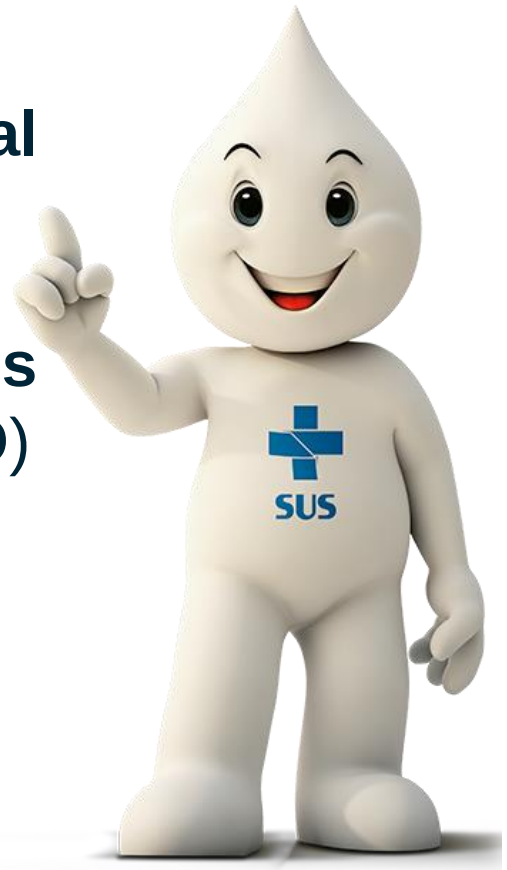
All Butantan Institute production is sent to PNI – Ministry of Health, in order to comply with national demand

Brazilian Immunization Programe (PNI)

- The National Immunization Program (PNI) in Brazil **is an international reference for public health policy**

- It was **created in 1973**, and the **program seeks social inclusion**

- **All Brazilians have free access to all vaccines** recommended by the World Health Organization (WHO)



GMP Certifications – ANVISA/WHO

- **ANVISA** (National Health Surveillance Agency) - NRA

- Butantan has **GMP certifications** for **API** (active pharmaceutical input) for **vaccines** and **sera** lines

- Butantan has **GMP certifications** for two pharmaceutical forms: **solutions** and **suspensions** of sterile small volume parenteral solutions

- Waiting for WHO inspection – PQ Influenza Seasonal Vaccine

Butantan Institute: hyperimmune sera *portfolio*

5 snake antivenoms

spider antivenom

scorpion antivenom

caterpillar antivenom

diphtheria antitoxin

tetanus antitoxin

botulism AB antitoxin

botulism E antitoxin

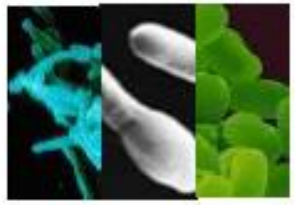
rabies antiviral

13 immunoglobulins

400,000 vials/year

Butantan Institute: vaccines *portfolio*

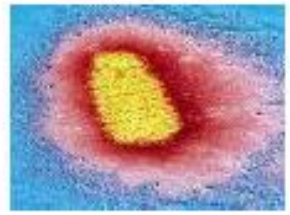
6 vaccines of the national calendar
150 million doses/year



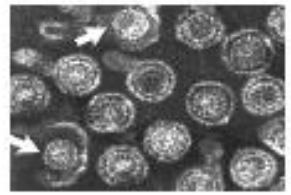
DTP
Diphtheria-Tetanus-Pertussis
(celular)

dT
Diphtheria-Tetanus
(for adults)

DT
Diphtheria-Tetanus
(for children)



rabies



hepatitis B

Influenza

Ongoing:
Dengue, Pandemic Influenza, Quadrivalent Influenza, rBCG, DTPHB, HPV, DTPa, HepA, mAbs

Alternative methods - challenges

- **Human resources: team training** (multidisciplinary training including management and innovation), **dedicated group to AM, and talent setting** (attractive remuneration).

- **Infrastructure: academia-industry interaction** (transfer and commercialization of technologies, import process)**, technological support structures** (reference centers) **and technological services** (conformity assessment).

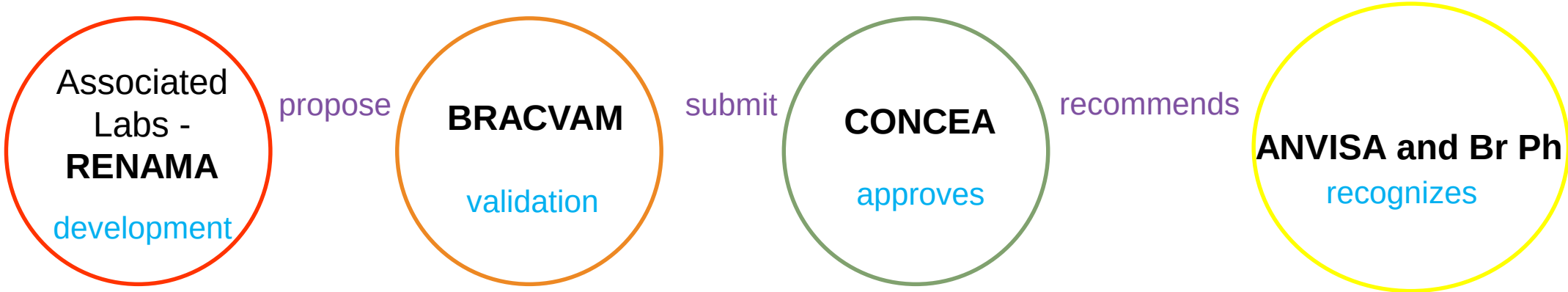
- **Investments: financing** (irregularity, discontinuity) **and investment in R&D&I** (tax exemption and import).

Source: Agência Brasileira de Desenvolvimento Industrial, 2012.

Alternative methods

- Regulatory approval !!!

Alternative methods – Regulatory proposal



3Rs iniciatives in Butantan Institute

Alternative methods can be defined as any method that can be used to replace, reduce or refine the use of animal in biomedical research, testing or teaching.

In vitro test (RFFIT) in rabies serum - Replacement

J Virol Methods. 2008 Dec;154(1-2):7-13. doi: 10.1016/j.jviromet.2008.09.010. Epub 2008 Oct 30.

Validation of a virus neutralization potency test in BHK-21 cells for rabies immunoglobulins in a two-center study.

de Moura WC[1], Gallina NM, Fuches RM, Romijn PC, Leite JP.

Farmacopeia Brasileira

Volume 2 - Monografias

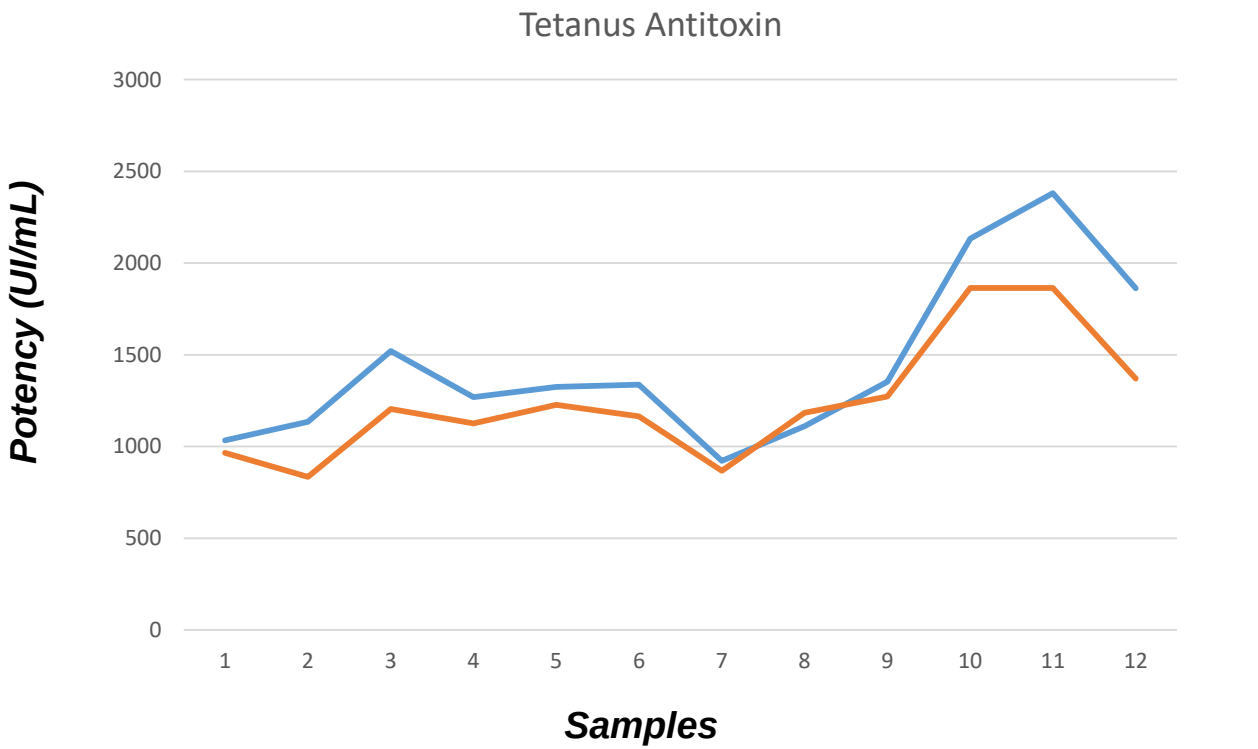
Método de soroneutralização de vírus rábico em células BHK_{21}

5ª edição

Brasília
2010

Save 600 mice per batch

Tetanus antitoxin potency by ToBI test - Replacement



Correl = 0.94

Farmacopeia Brasileira

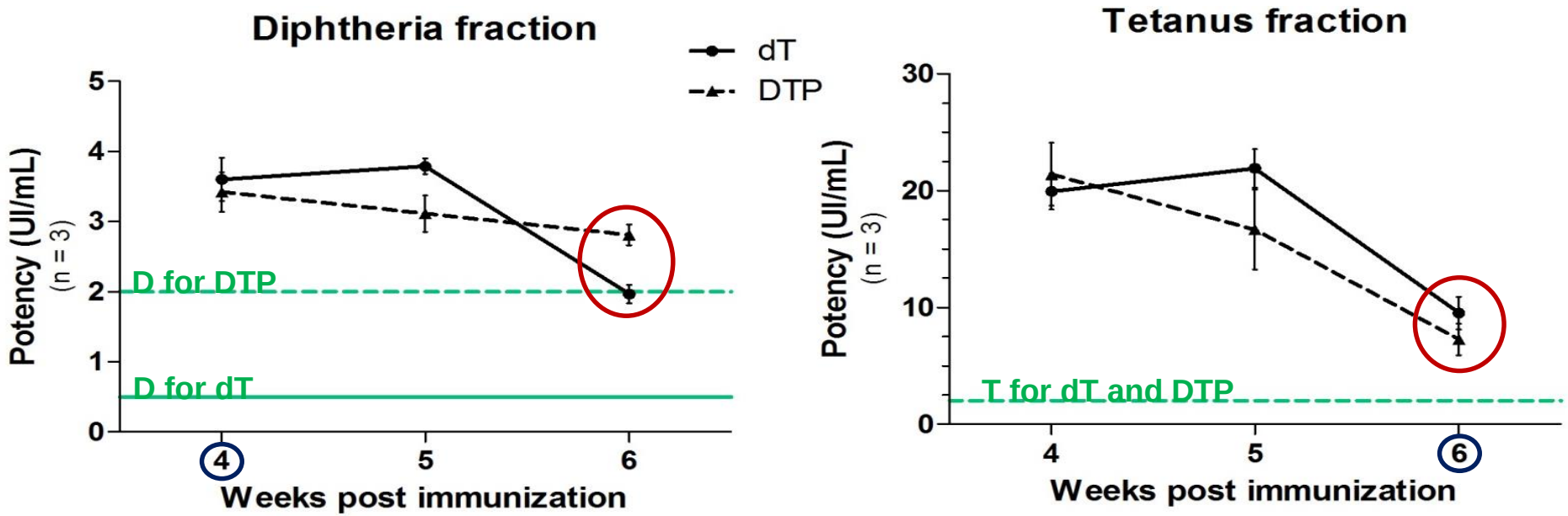
Volume 2 - Monografias

5ª edição

Brasília
2010

Save 330 mice per batch

Single bleeding for both fractions – Reduce/Refinement

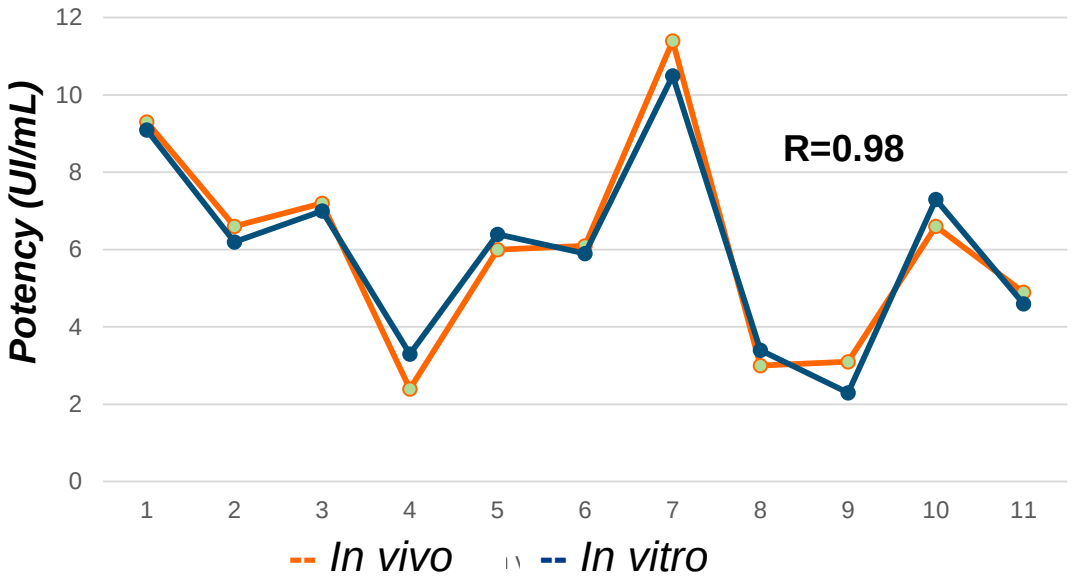


Animal reduction proposal: bleeding at 6 weeks for both D and T fractions.

Change Control CM/CB-00124/18 approved in 07 may 18

Save 6 guinea-pig per batch

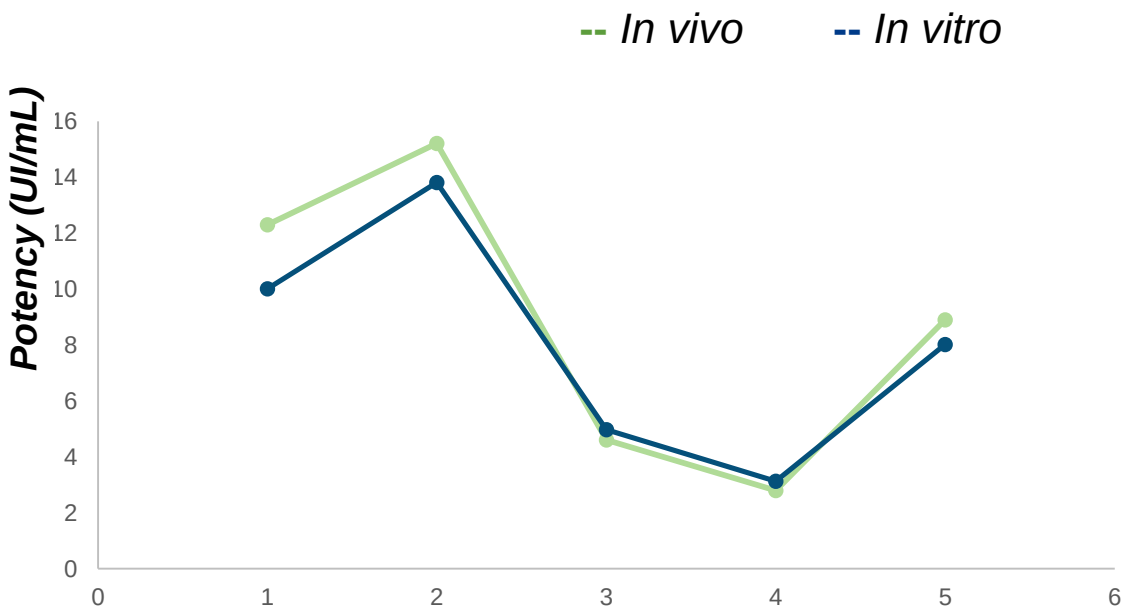
dT, DT, DTP, and DTPHB vaccines: Diphtheria fraction potency analysis *in vitro* and *in vivo* - *Replacement*



Correl = 0.98

Save 88 guinea-pig per batch

dT, DT, DTP, and DTPHB vaccines: Tetanus fraction potency analysis *in vitro* and *in vivo* - *Replacement*

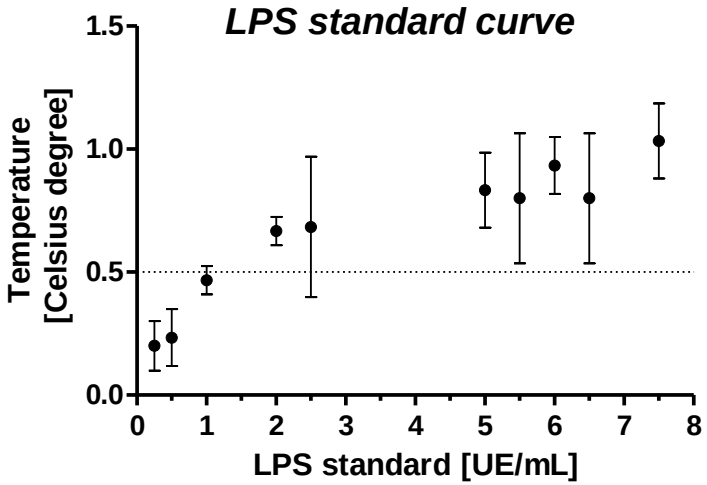


Correl = 0.99

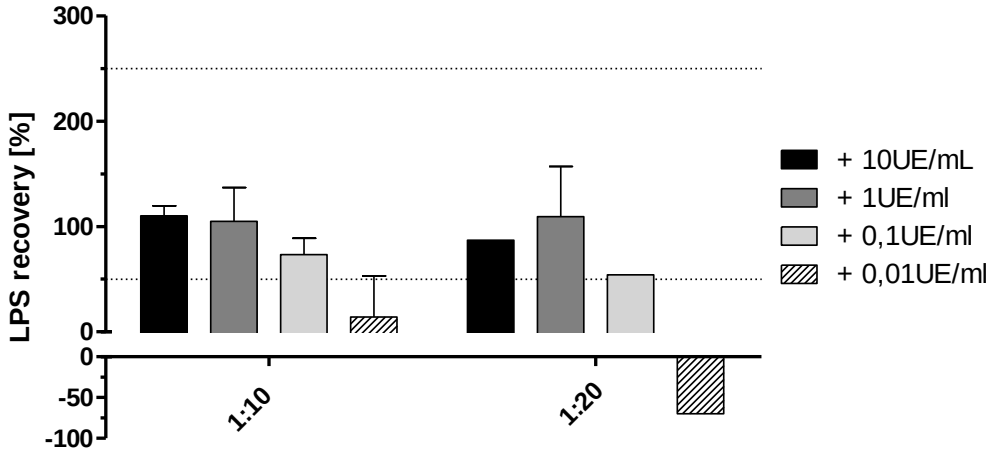
Save 220 mice per batch

LAL method for endotoxin quantification in hyperimmune sera - Replacement

In vivo

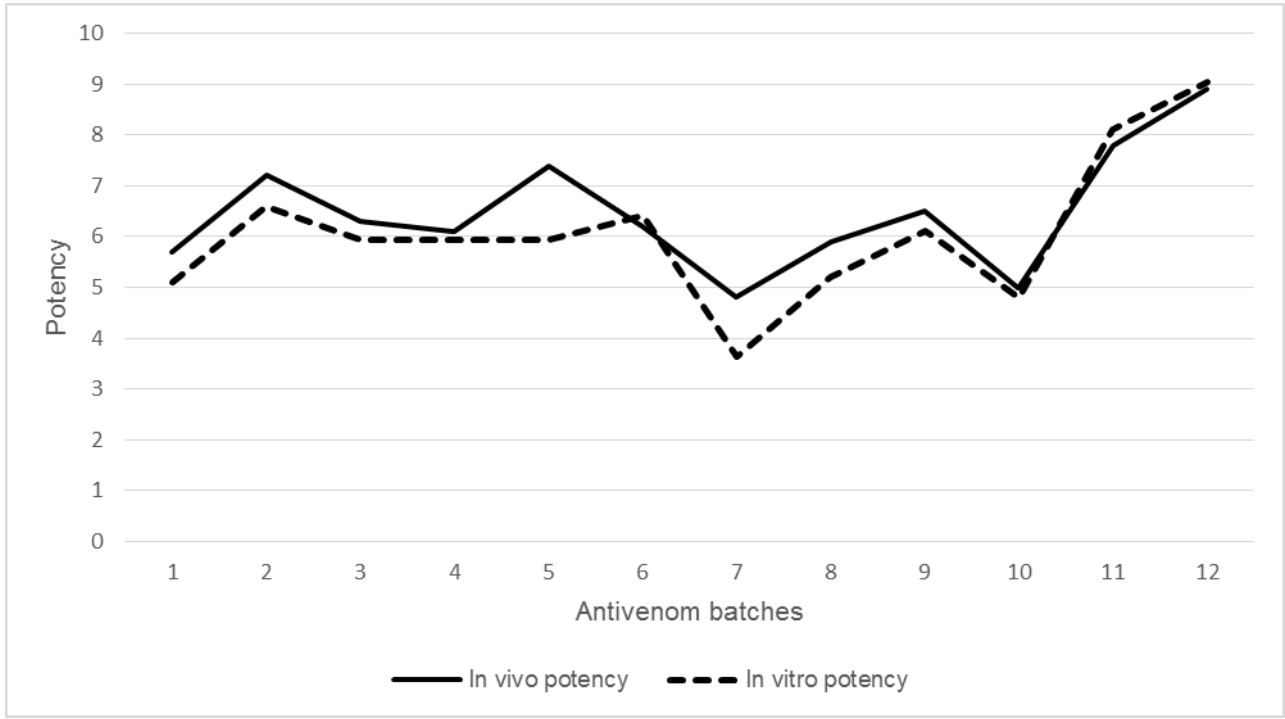


In vitro - LAL



Save 6-16 rabbits per batch

Bothrops jararaca sera potency by ToBI test - Replacement



Correl = 0.93

Save 330 mice per batch

Other 3Rs iniciatives in Butantan Institute

Replacement

On going in development/internal validation phase:

MAT – Monocyte Activation Test (consortium by RENAMA);

Diphteria sera potency by ToBI test;

Rabies potency test by ELISA (glycoprotein/anti-glycoprotein)

Abnormal toxicity test banishment (already done for Influenza vaccine – 70 millions doses per year); on going for other vaccines already registered (DTP, DT, dT, HepB)

Other 3Rs iniciatives in Butantan Institute:
Refinement

Previous anesthesia to intracerebral challenge for rabies and pertussis vaccines tests

Change Control CM/CB-00134/18 approved in 14 ago 18 and just implemented

End point to tetanus, rabies and pertussis tests

Change Control CM/CB-00116/18 approved in 23 march 18 and just implemented

Other 3Rs iniciatives in Butantan Institute:
Refinement

Pool for pyrogen and potency tests in antitoxins bulk ready-to-fill

Change Control CM/CB-00131/18 approved in 29 june 18 and just implemented

Previous anesthesia to bleeding guinea-pigs immunized for dT, DT and DTP vaccines

Euthanasia by isofluorane in mice

OBRIGADA!

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Diphtheria and Tetanus combined vaccine potency by ToBI

Alternative methods recommended by Eur Ph and WHO – determination of Ab titration (potency):

- **T fraction: ELISA and/or ToBI test**
- **D fraction: ELISA and/or Vero cell tests**

Our propose:

- Both fractions titration by **ToBI test**

In vitro potency test of vaccines: dT, DT, DTP and DTPHB + diphtheria and tetanus toxoid

- **ToBI test (Toxin Binding Inhibition Test)**

➢ Hendriksen et al, *J Biol Stand*, 1988. The toxin binding inhibition test as a reliable in vitro alternative to the toxin neutralization test in mice for the estimation of tetanus antitoxin in human sera

➢ **Similar principle of the *in vivo* potency test**

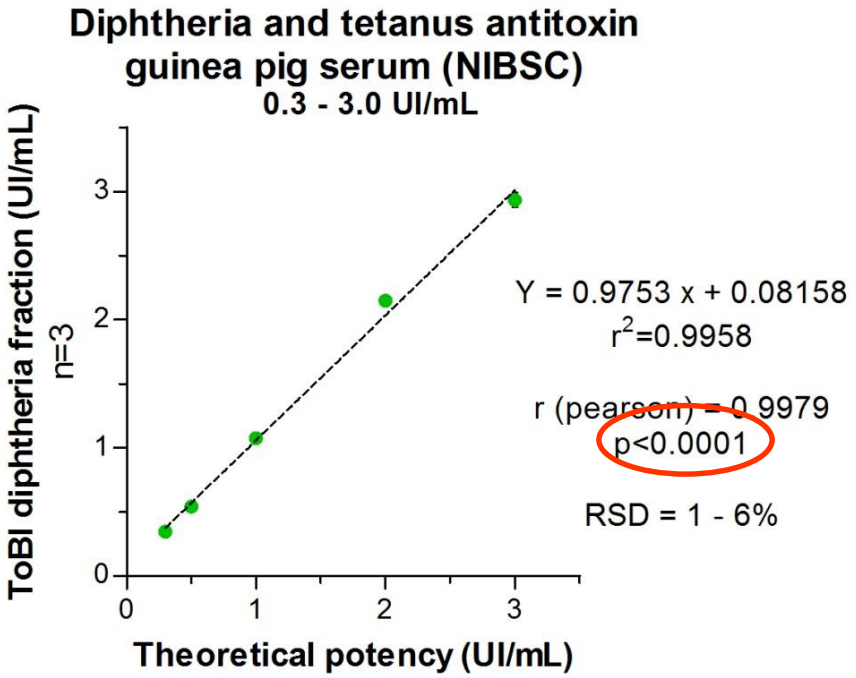
Decrease of 200 mice per batch of each vaccine and 400 mice per batch of anti-tetanus serum.

dT, DT, DTP, and DTPHB vaccines: selectivity

	Sample + toxoid	Sample + both (tetanus and diphteria) toxoid
DTPHB	**3.13 UI/mL**	**3.18 UI/mL**
	100%	101.6%
DTP	**4.97 UI/mL**	**5.14 UI/mL**
	100%	103.4%
Tetanus Toxoid	**8.01 UI/mL**	**7.24 UI/mL**
	100%	90.4%
dT	**10.01 UI/mL**	**10.1 UI/mL**
	100%	100.1%
DT	**13.81 UI/mL**	**14.48 UI/mL**
	100%	104.8%
Tetanus antitoxin NIBSC	**10.38 UI/mL**	**10.58 UI/mL**
	100%	102%

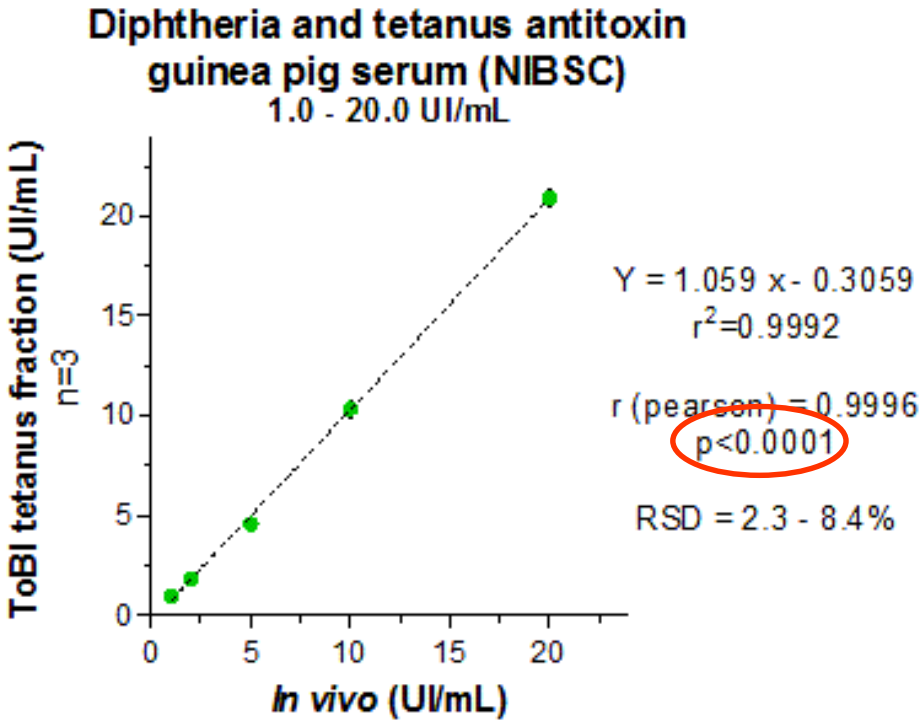
✓ ***All results between 80-120%***

dT, DT, DTP, and DTPHB vaccines: linearity Diphtheria fraction



Parameter	Specification	Experimental Results
Correlation coefficient (r)	≥ 0.950	0.9979
Y-intercept	---------	0.0816
slope	---------	0.9753
Coefficient of determination (r^2)	≥ 0.950	0.9958
Relative Standard Deviation (RSD)	≤ 20%	1 to 6%

dT, DT, DTP, and DTPHB vaccines: linearity Tetanus fraction



Parameter	Specification	Experimental Results
Correlation coefficient (r)	≥ 0.950	0.9996
Y-intercept	---------	- 0.3059
slope	---------	1.059
Coefficient of determination (r^2)	≥ 0.950	0.9992
Relative Standard Deviation (RSD)	≤ 20%	2 to 8%

dT, DT, DTP, and DTPHB vaccines: repeatability Diphtheria fraction

Analyst I

	dT (n=7)	DT (n=7)	DTPHB (n=9)	Diphtheria Toxoid (n=8)	DTP (n=7)
Mean (UI/mL)	3.38	4.67	6.57	6.68	10.38
Standard Deviation	0.43	0.71	0.62	0.85	1.07
Relative Standard Deviation* (≤20%)	13%	15%	9%	13%	10%

Analyst II

	dT (n=3)	DT (n=3)	DTPHB (n=3)	Diphtheria Toxoid (n=3)	DTP (n=3)
Mean (UI/mL)	2.76	3.99	6.09	5.82	9.27
Standard Deviation	0.21	0.15	0.25	0.36	0.68
Relative Standard Deviation* (≤20%)	8%	4%	4%	6%	7%

dT, DT, DTP, and DTPHB vaccines: repeatability Tetanus fraction

Analyst I

	DTPHB (n=3)	DTP (n=3)	Tetanus Toxoid (n=3)	dT (n=3)	DT (n=3)
Mean (UI/mL)	3.13	4.97	8.01	10.01	13.81
Standard Deviation	0.27	0.68	0.64	0.14	0.26
Relative Standard Deviation* (≤20%)	9%	14%	8%	1%	2%

Analyst II

	DTPHB (n=3)	DTP (n=3)	Tetanus Toxoid (n=3)	dT (n=3)	DT (n=3)
Mean (UI/mL)	3.21	5.3	8.74	10.31	13.91
Standard Deviation	0.12	0.31	0.99	0.98	0.23
Relative Standard Deviation* (≤20%)	4%	6%	11%	10%	2%

dT, DT, DTP, and DTPHB vaccines: intermediate precision Diphtheria fraction

SAMPLES	ANALYSTS I + II			$\frac{\lvert \bar{X}(An\ II) - \bar{X}(An\ I) \rvert}{\bar{X}(An\ I)} \times 100$
	Mean (UI/mL)	SD	RSD* (≤20%)	(≤20%)
dT	3.19	0.47	15%	18%
DT	4.46	0.67	15%	15%
DTPHB	6.45	0.58	9%	7%
Diphtheria Toxoid	6.45	0.84	13%	13%
DTP	10.05	1.07	11%	11%

* RE 899/2003 (ANVISA): RSD ≤ 5%)

dT, DT, DTP, and DTPHB vaccines: intermediate precision Tetanus fraction

SAMPLES	ANALYSTS I + II			$\frac{\lvert \bar{X}(An\ II) - \bar{X}(An\ I) \rvert}{\bar{X}(An\ I)} \times 100$
	Mean (UI/mL)	SD	RSD* (≤20%)	(≤20%)
DTPHB	3.17	0.19	6%	3%
DTP	5.13	0.51	10%	7%
Tetanus Toxoid	8.37	0.85	10%	9%
dT	10.16	0.65	6%	3%
DT	13.86	0.23	2%	1%

* RE 899/2003 (ANVISA): RSD ≤ 5%)

dT, DT, DTP, and DTPHB vaccines: accuracy Diphtheria fraction

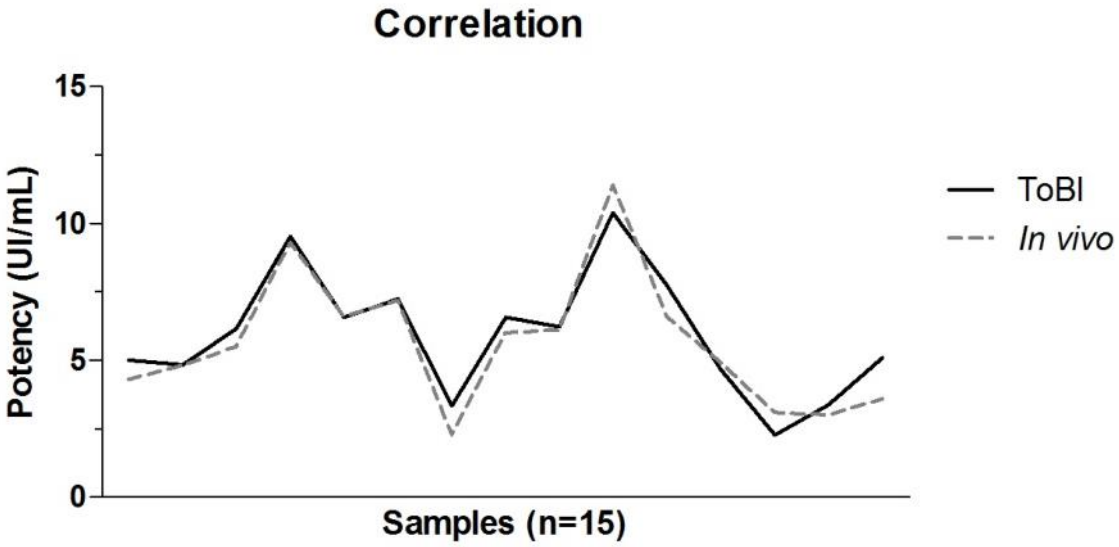
SAMPLES	$\bar{X}$ (ToBI) (UI/mL) n = 7	Official Result *In vivo* (UI/mL) n = 1	$\frac{\bar{X}\text{ (ToBI)}}{\text{Official Result}}$ x 100 (80 to 120%)
dT	3.38	3.0	113%
DT	4.67	4.9	95%
DTPHB	6.57	6.0	110%
Diphtheria Toxoid	6.68	6.6	101%
DTP	10.38	11.4	91%

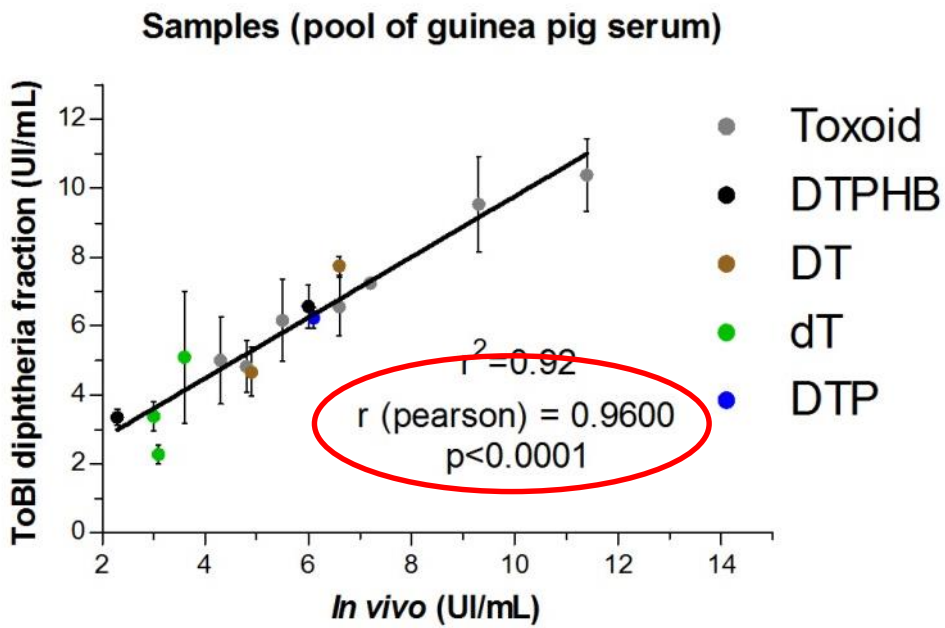
dT, DT, DTP, and DTPHB vaccines: accuracy Tetanus fraction

SAMPLES	$\bar{X}$ (ToBI) (UI/mL) n = 3	Official Result *In vivo* (UI/mL) n = 1	$\frac{\bar{X}\text{ (ToBI)}}{\text{Official Result}} \times 100$ (80 to 120%)
DTPHB	3.13	2.8	112%
DTP	4.97	4.6	108%
Tetanus Toxoid	8.01	8.9	90%
dT	10.01	12.3	81%
DT	13.81	15.2	91%

dT, DT, DTP, and DTPHB vaccines: Diphtheria fraction potency analysis

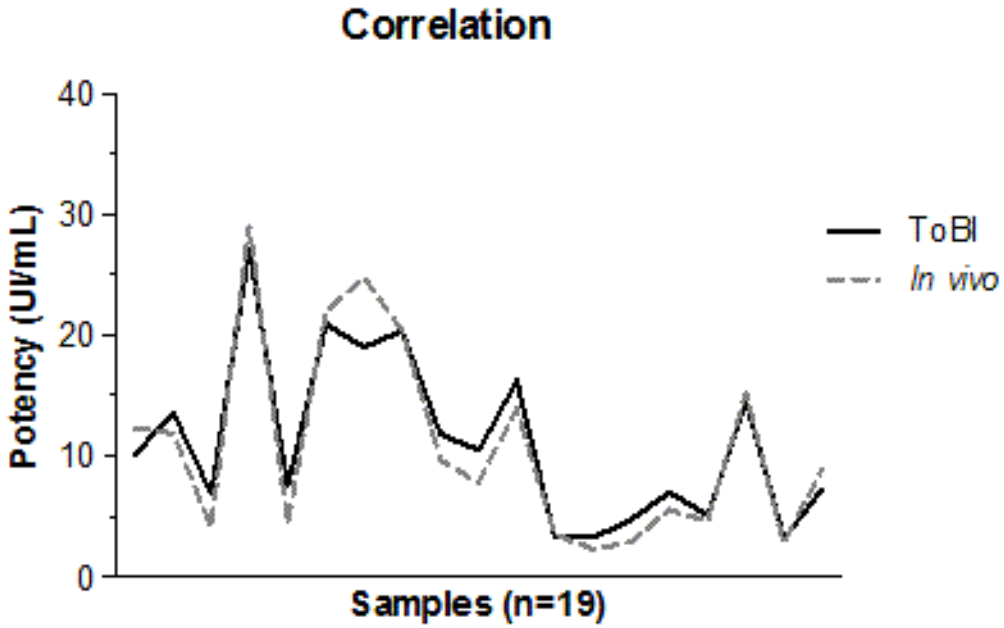
***in vivo* e *in vitro* correlation**

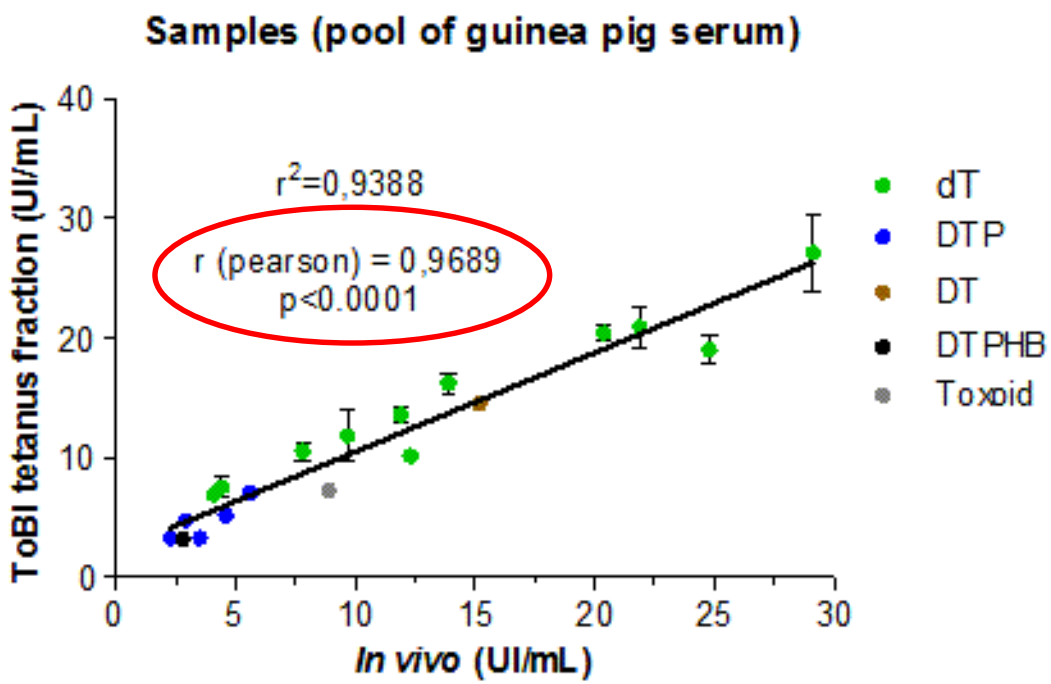




dT, DT, DTP, and DTPHB vaccines: Tetanus fraction potency analysis

***in vivo* e *in vitro* correlation**





Conclusion after internal validation

✓ *Diphtheria and Tetanus combined vaccine* potency by ToBI

- ✓ **Internal validated ToBI tests presented suitable** to estimate the **potency not only to tetanus fraction but ALSO to diphtheria fraction in combined vaccines**.
- ✓ **Our data met all acceptance validation criteria**, as specificity/selectivity, linearity, precision (repeatability and intermediate precision), accuracy, and *in vitro* and *in vivo* concordance showed strong correlation (**R=0.96**) for **both D and T components**.
- ✓ The **high relevance of these data for replacement of animal testing strongly impacts the routine of Quality Control**, turning the analyses **faster, accurate and cheaper** and, more importantly, **saving the lives of hundred of animals per year**.

Biotech QC team:

Thank you!

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