INSTITUTO BUTANTAN

2nd PAN-AMERICAN Conference for Alternative Methods

August 23-24, 2018
Rio de Janeiro

Butantan experience on the *in vitro* alternatives for hyperimmune sera analysis: safety and potency tests.

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Hyperimmune sera

- **are polyclonal antibodies** produced by immunized animals, mainly horses;

- **used for human treatment** after of **animal venoms poisoning**, and also for **bacterial and viral infections** (which **triggers humoral immune response**);

- **Main producers are public** companies:

  - **Clodomiro Picado** Institute (Costa Rica); **Biological Institute** (Argentina);
  - in Brazil: - **Butantan Institute**,
    - **Vital Brazil Institute**,
    - Ezequiel Dias Foundation (**FUNED**),
    - Center for immunobiological production and research (**CPPI**).

Butantan Institute: 116 year-old of history

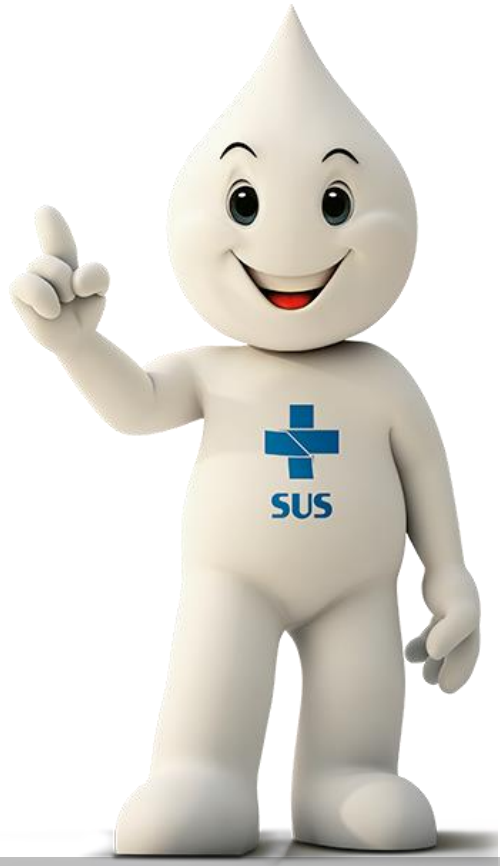
www.butantan.gov.br

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

<u>All Butantan Institute production is sent to PNI – MoH in order to comply with national demand</u>

- All Brazilians have free access to all vaccines

The Butantan is committed to public health



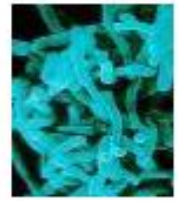
Butantan Institute: hyperimmune sera *portfolio*

5 snake antivenoms

spider antivenom

scorpion antivenom

caterpillar antivenom



diphtheria antitoxin

tetanus antitoxin

botulism AB antitoxin

botulism E antitoxin

rabies antiviral

GMP Certifications – ANVISA, and WHO (in request)

Current scenario

✓**Brazilian Pharmacopoeia: specific monographies for hyperimmune sera for human use;**

✓ Tests: physico-chem, microbio (sterility and **pyrogens**), and bio assays (ID and **ED50 - potency**).

✓Scientific literature and individual approaches indicate **opportunities for alternative methods**

AM for pyrogens on rabbits

✓LAL (Limulus amebocyte lysate): chromogenic and kinetic turbidimetric:

Pro: technically simple, fast, cheap;

Con: only for endotoxins, product appearance could interfere on chromogenic measurement;

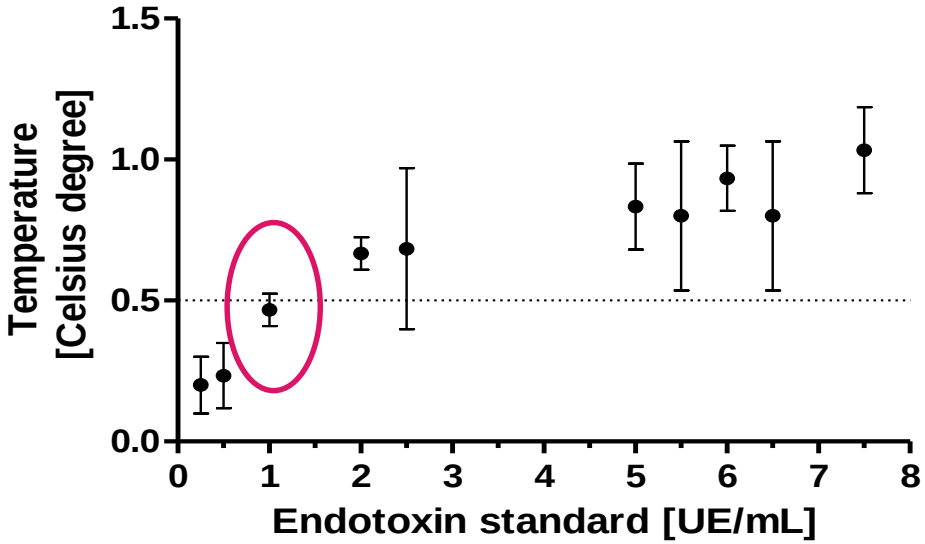
✓MAT (Monocyte activation test):

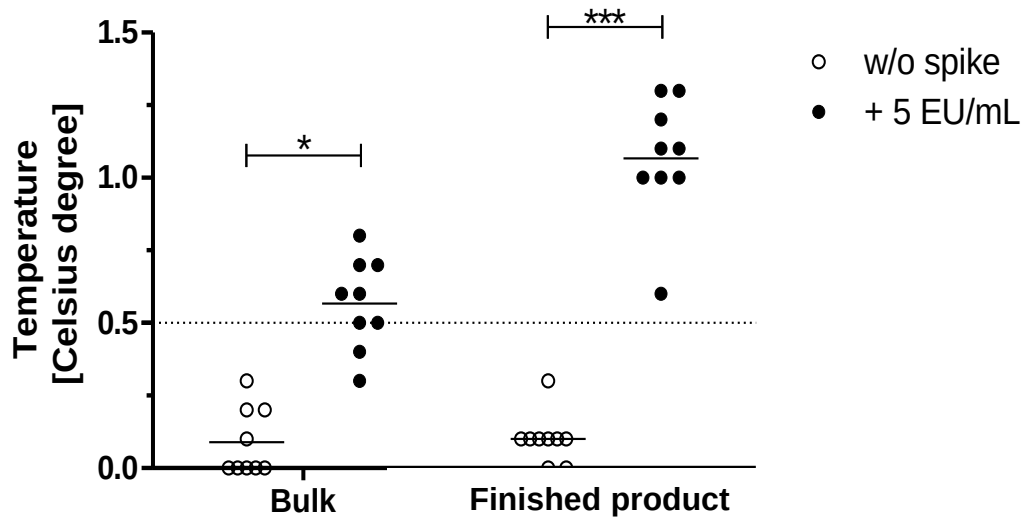
Pro: detects pyrogens, technically simple;

Con: expensive, blood-dependent, and is waiting for Brazilian acceptance.

Suitability of the kinetic turbidimetric LAL method for endotoxin quantification in hyperimmune sera

In vivo assays





- Standard endotoxin: E. coli Strain O55:B5 (Lonza)

✓***in vivo*** **quantification limit: 1EU/mL;**

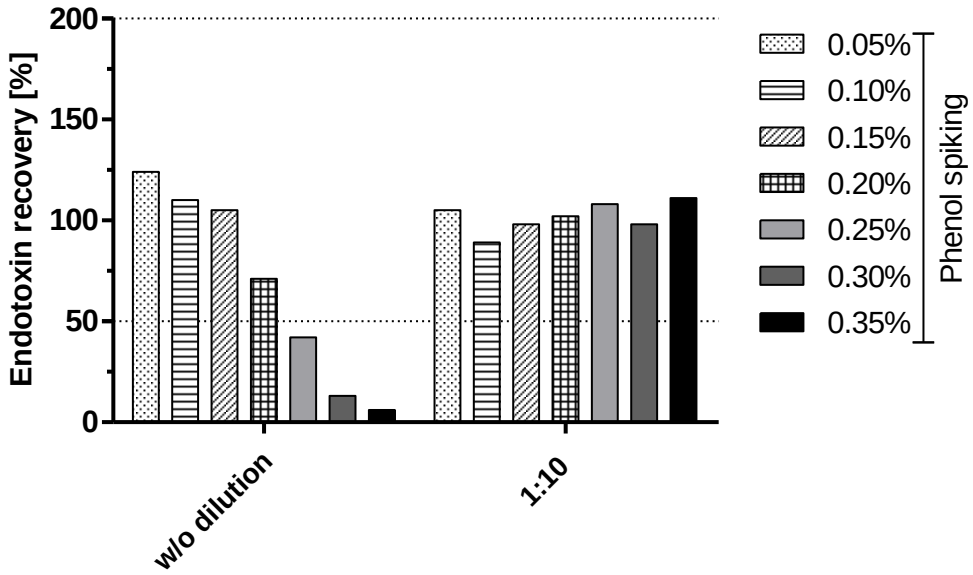
Suitability of the kinetic turbidimetric LAL method for endotoxin quantification in hyperimmune sera

In vitro assays

✓Sample prep: no need of sample heating;
✓MVD (maximum valid dilution): 1:30;
✓Sample dilution: bulk 1:20; finished product 1:10.

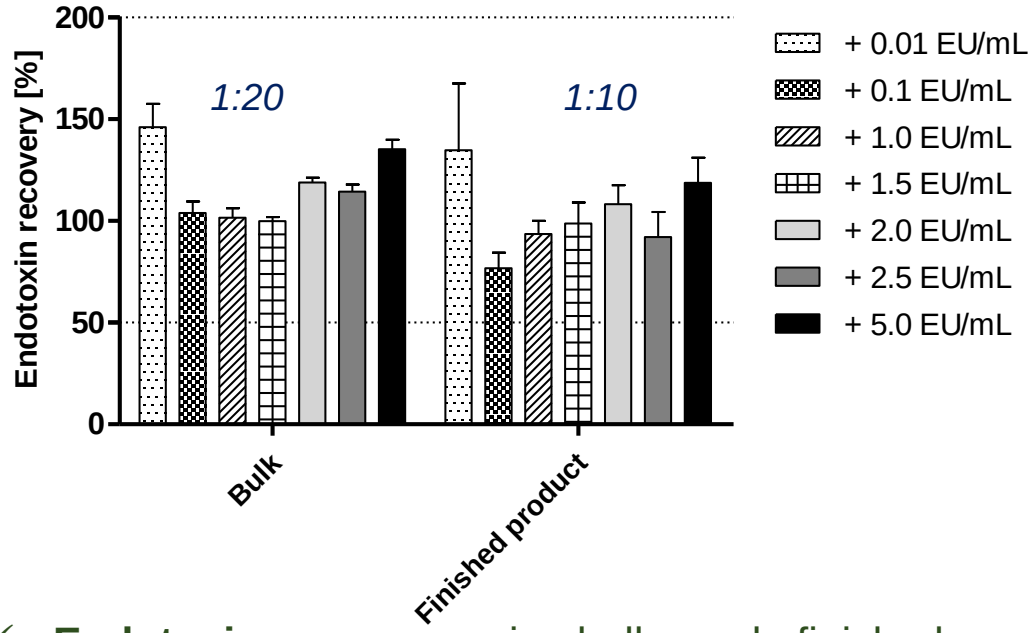
Phenol interference evaluation

Phenol specification: <0,35%



✓ **Phenol** reagent, as preservative of sera, **did not interfere on endotoxin recovery** at optimum dilution sample;

Endotoxin recovery

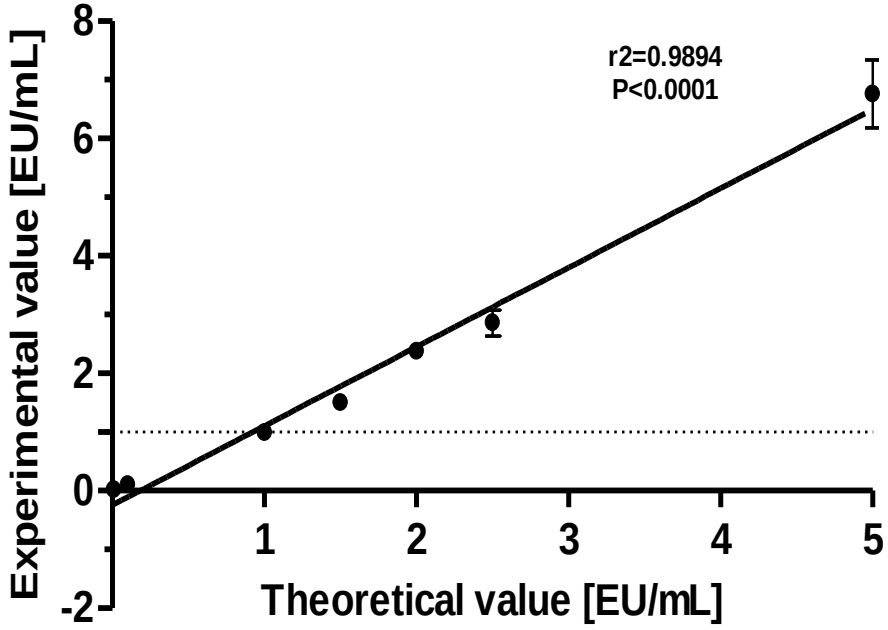


✓ **Endotoxin recovery** in bulk and finished sera samples **was high and is within the compendial specification (50 to 200%);**

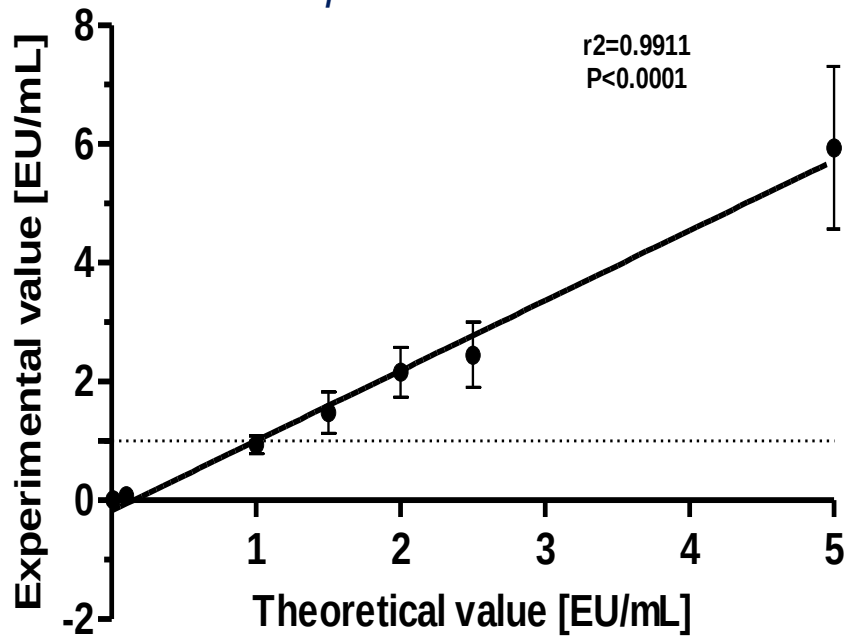
Suitability of the kinetic turbidimetric LAL method for endotoxin quantification in hyperimmune sera

Linearity of LPS-spiked recovery in sera samples

Bulk



Finished product



✓ **Linearity of endotoxin recovery** for bulk and finished product **presented R^2>0.98 from 0.01 to 5 EU/mL.**

AM for potency *in vivo*: ToBI test: Toxin-binding Inhibition test**

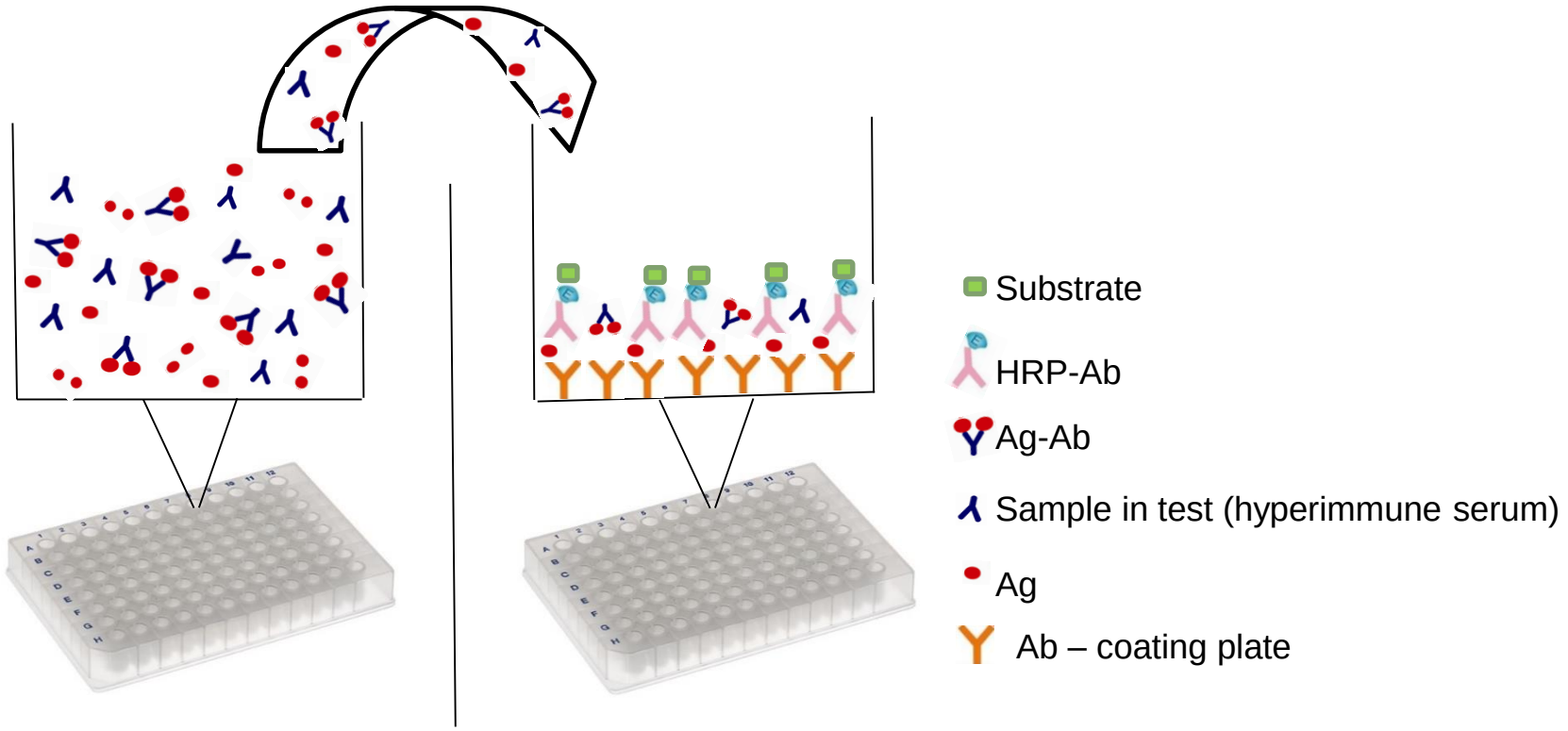
Similar principle of the *in vivo* potency test

Goal: ***Quantify*** *high affinity antibodies (**neutralizing Ab**)*

Suitable for toxins in general which triggers humoral response.

**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.

Transference after serum neutralization step

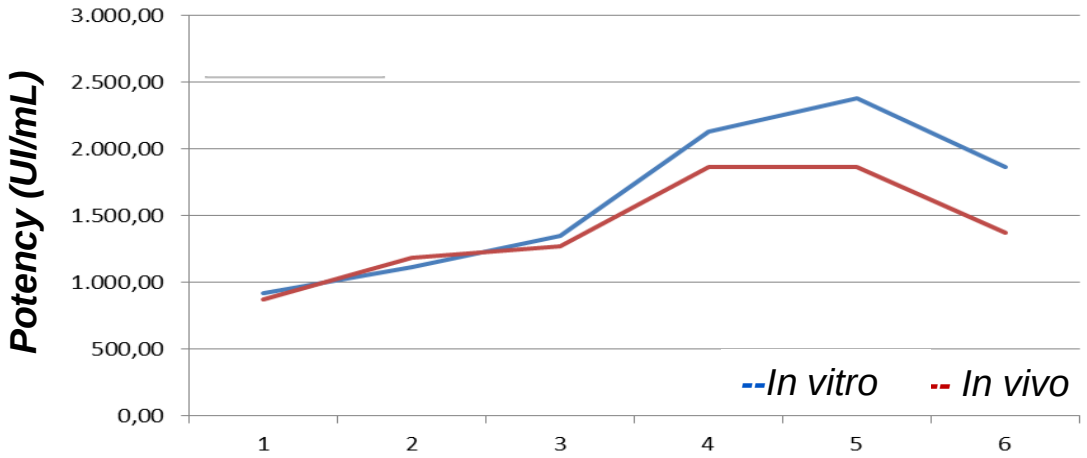


Step 1: serum neutralization

Step 2: ELISA assay

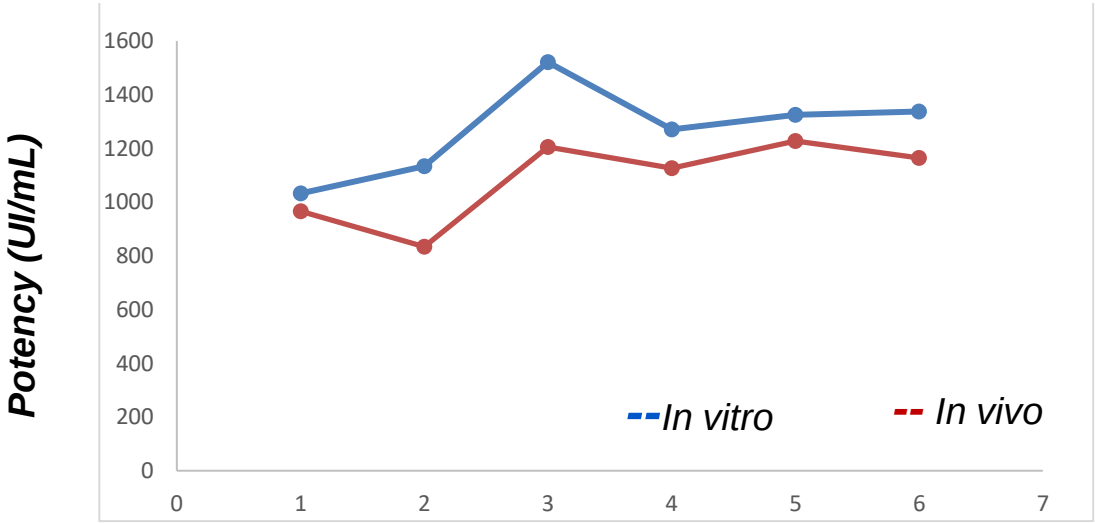
Tetanus antitoxin potency by ToBI : *in vitro* and *in vivo correlation*

Bulk



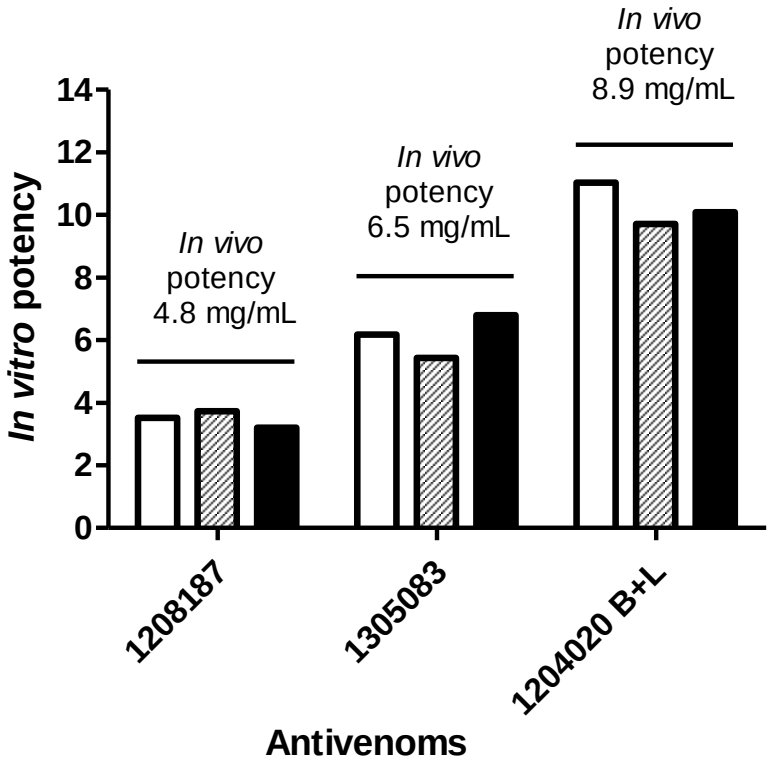
Samples	ToBI	In vivo	Correl
1	921,1	868,0	0.95
2	1111,3	1184,4	
3	1352,5	1273,3	
4	2134,4	1864,0	
5	2381,1	1864,0	
6	1863,3	1371,0	

Final product

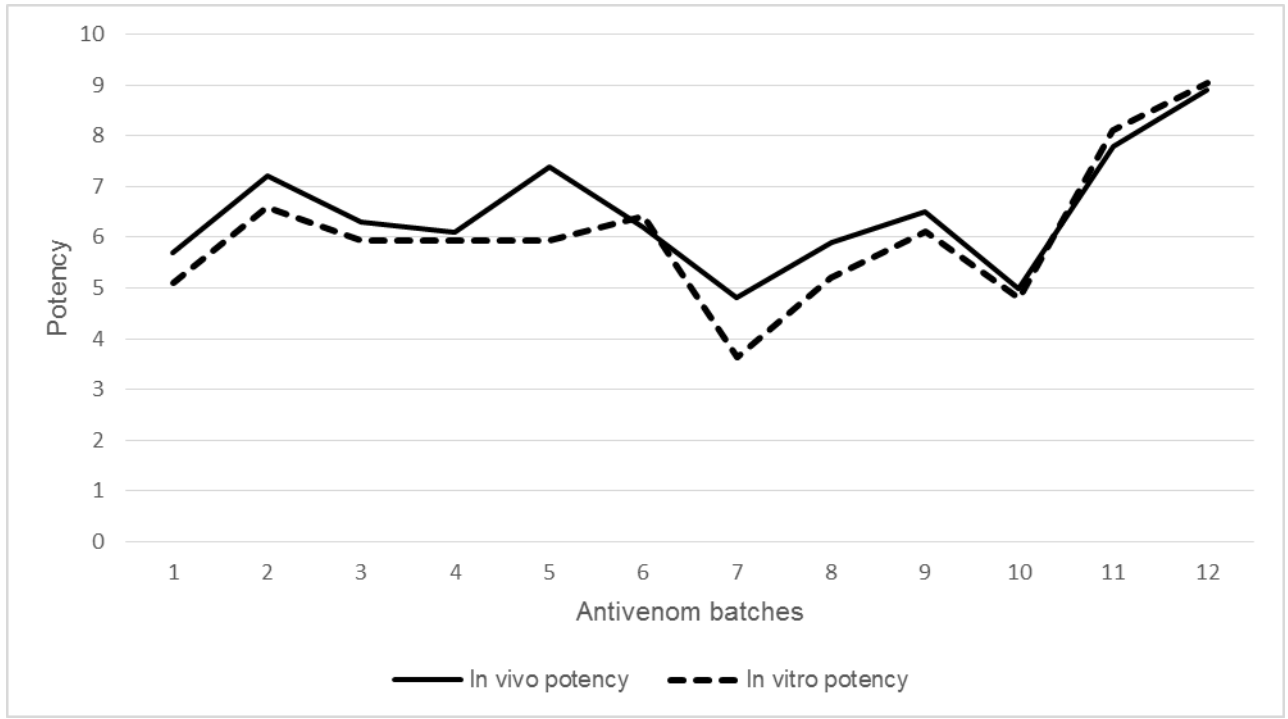


Samples	ToBI	In vivo	Correl
1	1032,7	965,5	0.8
2	1133,8	833,5	
3	1520,8	1205	
4	1270	1126	
5	1325	1227	
6	1337	1164	

Bothrops jaraca sera potency by ToBI test - Replacement



Correl = 0.93



Process improvement– impact of AM insertion in the QC

- All sera, pyrogen analysis:

	In vivo*	**In vitro LAL****
Run test -total time	**8h** (pre-test and test)	**~1h**
Animals employed	**3 rabbit/product** (+5 rabbit/product: if retested)	NA

- Hyperimmune sera: potency:

*** Per product batch**

	In vivo*	**In vitro (ToBI)**
Run test -total time	**~48h**	**8h**
Animals employed	**220 mice** (bulk and finished prod)	**96w microplate (5 sera)**
Reprodutibility	intermediate	high
Variability	high	Lower than *in vivo*

Other 3Rs initiatives at Butantan Institute

- **Diphtheria antitoxin sera potency (ToBI test);**
- **Potency of D and T fractions of combined vaccines (ToBI test);**
- **Abnormal toxicity test banishment (already done for Influenza vaccine – 60 millions doses per year).**

To be discussed

6Rs:

-**Replacement**

-**Refinement**

-**Reduction**

-**Read-across:** test applicabilty for sera from other products

-Relevance

-Roadmaps: communications, RENAMA partnership, data publication, and CONCEA consultation:

How to optimize visibility of biologicals and move forward on AM approval.

Biotech Quality Control team

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