

Alternative Methods to Animal Testing of Vaccines and Hyperimmune sera: Butantan Institute Experience

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- Use of laboratory animals:
 - Expensive activity
 - Needs specialized workers
 - Takes long to achieve valid results
 - Poor reproducibility
 - Sometimes not completely relevant
 - Ethical concern.
- General feeling in favor of reducing and replacing the use of animals in addition to the technological development of viable alternatives.

- Some international compendiums, European Pharmacopoeia, e.g. consider the alternatives for quality control of several biological products.
- In Brazil - only few approaches in that field.
 - Brazilian Pharmacopoeia mainly *in vivo* assays for quality control of biologicals and medicines.

- Butantan Institute

– For the last years it has been the one of largest Brazilian biologicals producer

Vaccines	Hyperimmune Sera	Biopharmaceuticals
Diphtheria	Rabies	Pulmonary surfactant
Tetanus	Diphtheria	Monoclonal antibodies
Pertussis	Tetanus	
Hepatitis B	Anti-venom	
Influenza		
Rabies		

- Very large number of animals used in quality control
- Justification and encouragement for the research on alternatives

- Since 1999, Butantan has been investing on alternative assays, either in new assays development or in implementation of those already described
- Cornerstone
 - “Laboratório de testes *in vitro*” at Biological Control
Started in 2000/2001 from Dr Rugimar Marcovistz works

- Achievements

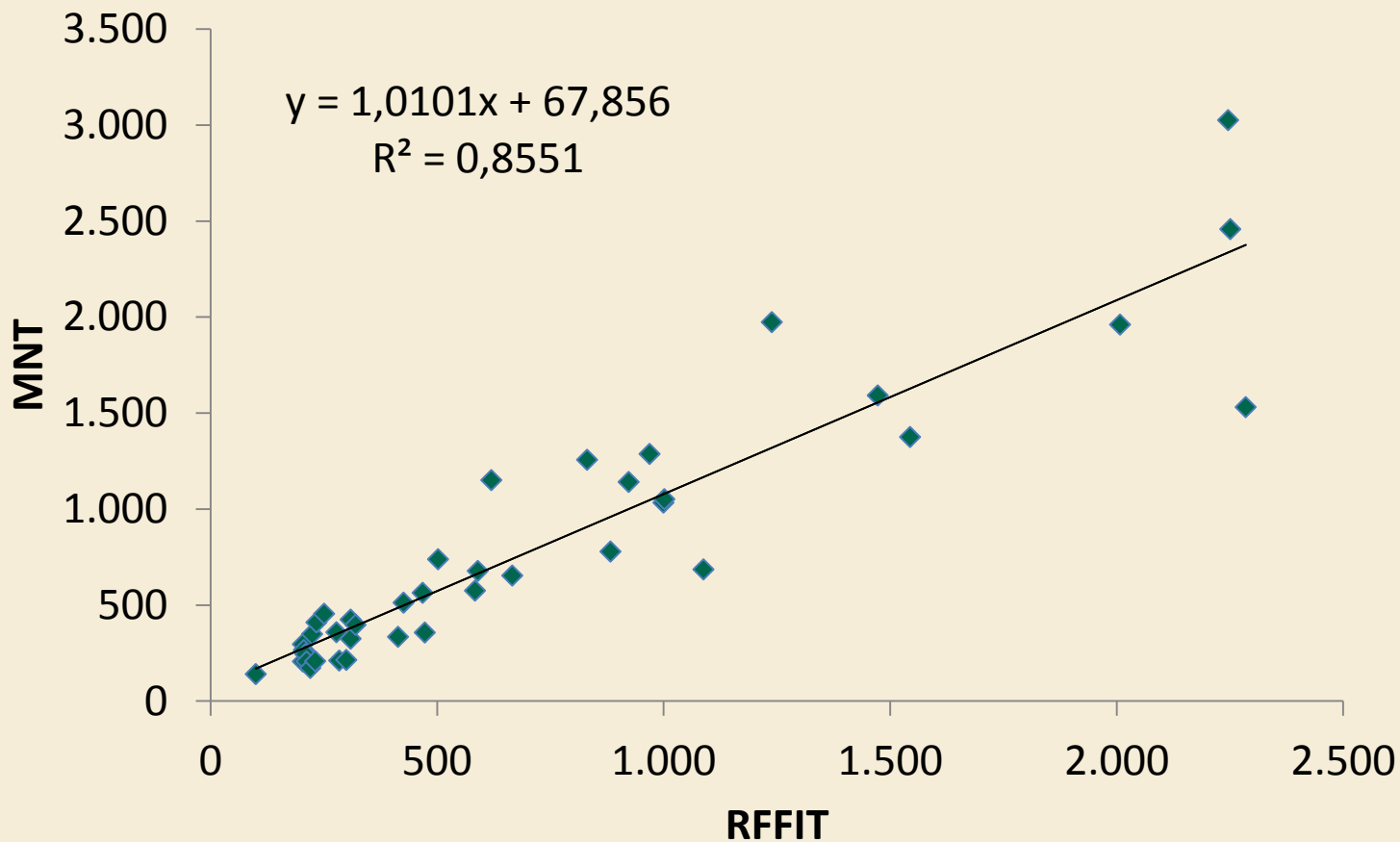
- Hepatitis B vaccine

- 2001-2004 -ELISA assay as a consistency test to assess vaccine activity – reduction by 95% of animals needed to lot release

- Rabies antiserum

- Rabies anti-serum – Mouse test: stressful and difficult
The joint effort of Butantan Institute and INCQS (Fiocruz) lead to the **total** substitution of the in vivo test and to the alternative in vitro test (RFFIT) publication in Brazilian Pharmacopoeia.

RFFIT x Mouse Neutralization assay for rabies anti-serum



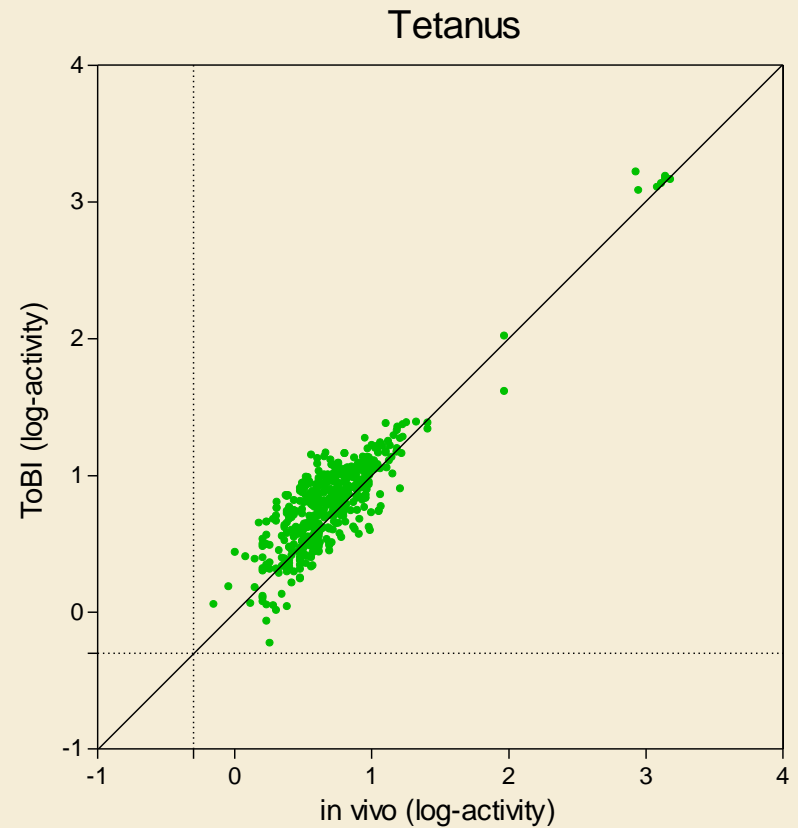
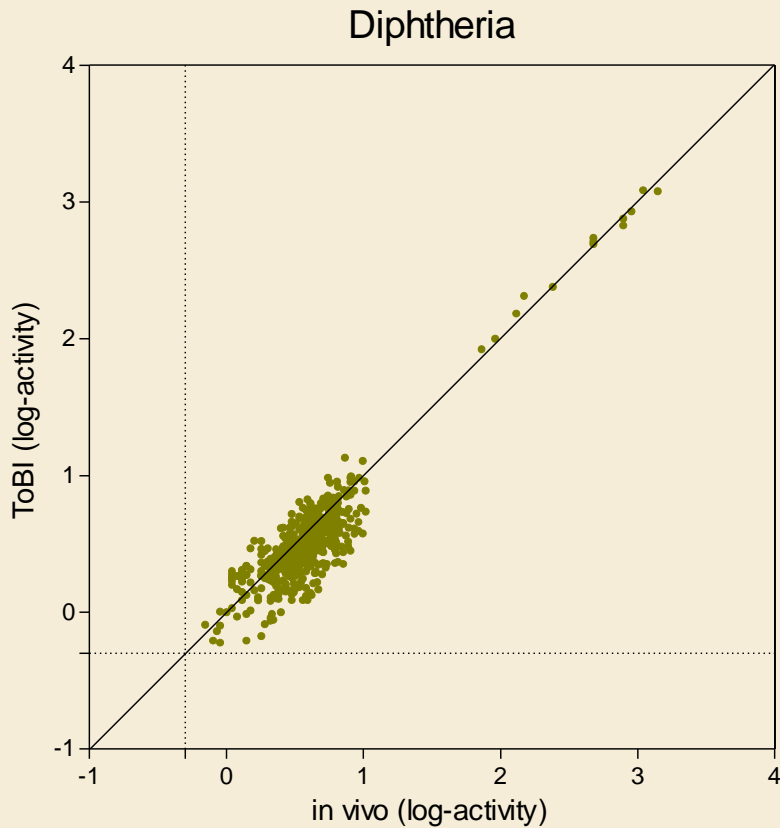
[J Virol Methods](#). 2008;154(1-2):7-13.
doi: 10.1016/j.jviromet.2008.09.010

- DT/DTP vaccine components
 - “American (NIH)” method → serology
 - “European” method → challenge
- Diphtheria/tetanus hyperimmune antiserum



Mice/guinea-pigs x ToBI test

- Started in year 2000 after Dr Marcovistz publications
- Accessory test to the regular *in vivo* serum titration addressing the serum dilutions to be tested
→ reducing the number of repetitions
- When fully implemented this test can reduce by 86% on average the number of animals.

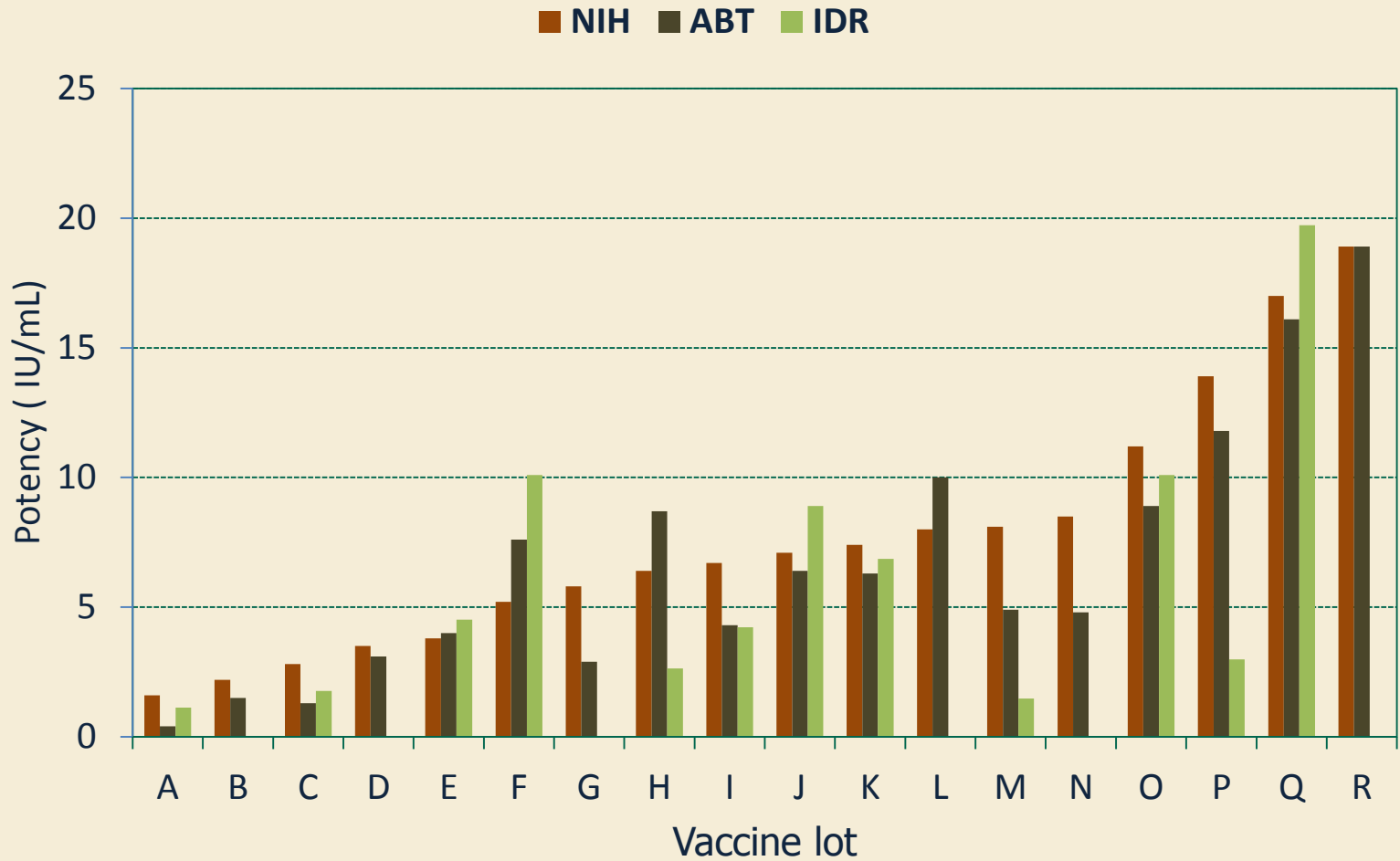


Biologicals, 43 (2015), 55-61; doi:10.1016/j.biologicals.2014.10.001

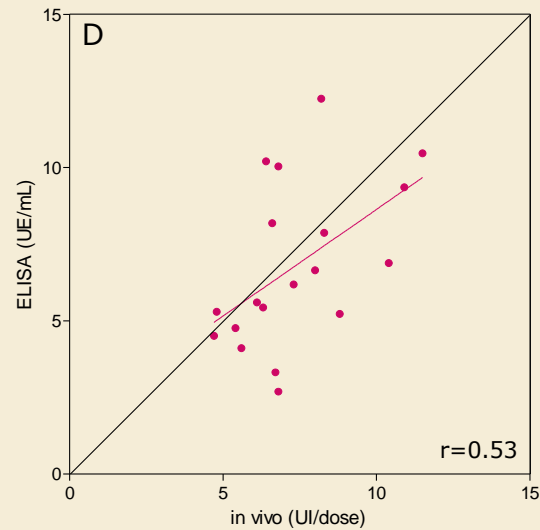
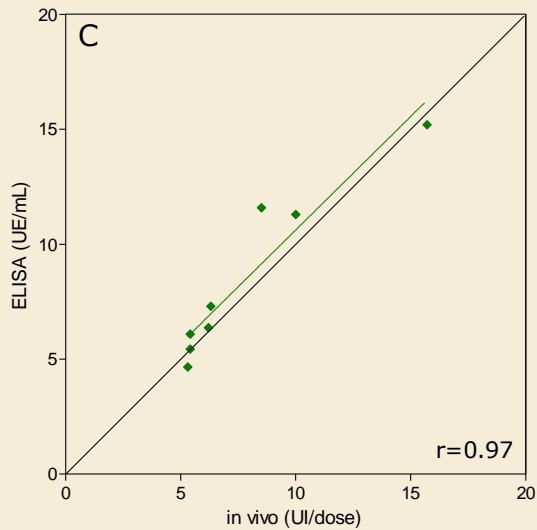
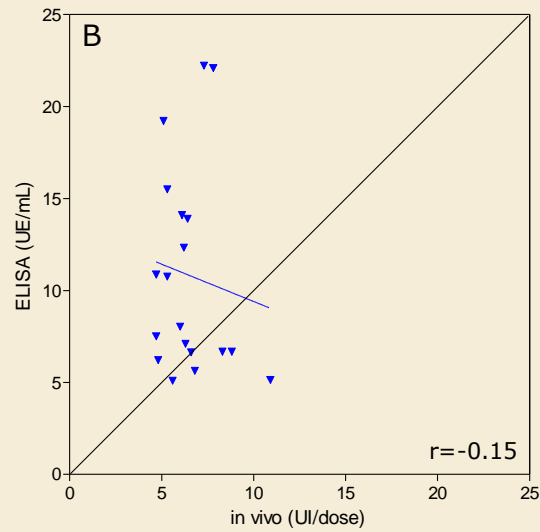
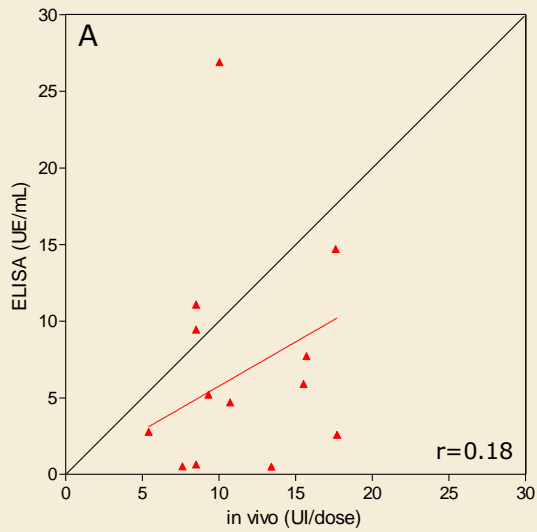
- Alternatives for rabies vaccine potency assay under development:
 - Serological test – reduces the number of animals and refine the results
 - Glycoprotein by SRD – consistency approach
 - ABT (antibody binding test) – reduces the number of animals

- Preliminary results:

NIH x ABT x SRD for rabies vaccine potency assessment



- Serological test to assess pertussis potency in combined vaccines
 - DTP immunized guinea-pigs
 - ELISA using *B. pertussis* 18323, purified PT or pertussis vaccine
 - Bleeding time: 28 or 42 days



- A. PT coated ELISA and 28th day bleed;
- B. 18323 *B. pertussis* coated ELISA and 28th day bleed;
- C. 18323 *B. pertussis* coated ELISA and 42nd day bleed;
- D. Pertussis vaccine coated ELISA and 28th day bleed.

- Diphtheria vaccine toxicity
 - Not totally new, but a gap in Butantan
 - alternative method leading to **total replacement** of guinea-pigs using Vero cells
 - serum free media → vaccine test completely without animals.

- Detection of bacterial endotoxins in hyperimmune sera by turbidimetric kinetic method as alternative to *in vivo* pyrogen test

Product	<i>In vivo</i> Assay	<i>In vitro</i> Assay	
		Results	Spike recover
Serum A	Fail	29,45 EU/mL	27%
		30,86 EU/mL	105%
		23,29 EU/mL	112%
		15,82 EU/mL	101%
		21,47 EU/mL	160%
		17,17 EU/mL	113%
Serum B	Pass	1,20 EU/mL	80%
		1,57 EU/mL	79%
		1,58 EU/mL	74%
		< 5,00 EU/mL	99%
		< 5,00 EU/mL	99%
		< 5,00 EU/mL	99%

- Ongoing studies:
 - Monocytes activation test (MAT) for total rabbit replacement for pyrogen test
 - Adventitious agents for vaccines produced in Vero cells
 - Other projects

- Researchers involved
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Thank you!

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