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

Wagner Quintilio, PhD
Scientific Researcher
• 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

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Hyperimmune Sera</th>
<th>Biopharmaceuticals</th>
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<tbody>
<tr>
<td>Diphtheria</td>
<td>Rabies</td>
<td>Pulmonary surfactant</td>
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<tr>
<td>Tetanus</td>
<td>Diphtheria</td>
<td>Monoclonal antibodies</td>
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<tr>
<td>Pertussis</td>
<td>Tetanus</td>
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<td>Hepatitis B</td>
<td>Anti-venom</td>
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<td>Influenza</td>
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<tr>
<td>Rabies</td>
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</table>
– 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

\[ y = 1.0101x + 67,856 \]

\[ R^2 = 0.8551 \]

• 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

Potency (IU/mL)

Vaccine lot

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 28\textsuperscript{th} day bleed;

B. 18323 \textit{B. pertussis} coated ELISA and 28\textsuperscript{th} day bleed;

C. 18323 \textit{B. pertussis} coated ELISA and 42\textsuperscript{nd} day bleed;

D. Pertussis vaccine coated ELISA and 28\textsuperscript{th} 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 kynetic method as alternative to *in vivo* pyrogen test

<table>
<thead>
<tr>
<th>Product</th>
<th><em>In vivo</em> Assay</th>
<th><em>In vitro</em> Assay</th>
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<tbody>
<tr>
<td></td>
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<td>Results</td>
</tr>
<tr>
<td>Serum A</td>
<td>Fail</td>
<td>29,45 EU/mL</td>
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<tr>
<td></td>
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<td>30,86 EU/mL</td>
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<td>23,29 EU/mL</td>
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<td>15,82 EU/mL</td>
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<td>21,47 EU/mL</td>
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<td>17,17 EU/mL</td>
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<tr>
<td>Serum B</td>
<td>Pass</td>
<td>1,20 EU/mL</td>
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<td>1,57 EU/mL</td>
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<td>&lt; 5,00 EU/mL</td>
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</tbody>
</table>
• 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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– Fábio Alessandro de Freitas
– Josana Kapronezai
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wagner.quintilio@butantan.gov.br

+55 (11) 2627-9450/9844