HISTAMINE SENSITIZATION TEST FOR ACELLULAR PERTUSSIS VACCINES

A review ofacellular pertussis vaccine safety test regulatory requirements
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Pertussis Toxin: A member of the “AB$_5$” Holotoxin Family

A Protomer (ADP-ribosyltransferase)

B Oligomer (Binding)


SANOFI PASTEUR
Acellular pertussis (aP) vaccine safety test regulatory requirements

- Safety tests are required by regulatory authorities to assure the absence of residual toxicity or reversion of pertussis toxoid to toxin in pertussis vaccines.
- The *in vivo* murine histamine sensitization test (HIST)
  - currently accepted regulatory method (JP, WHO, EU, CA, US) used to monitor residual pertussis toxin (PTx) activity in acellular pertussis vaccines.
  - Mice normally generally resistant to lethal effects of histamine
  - Pertussis toxin increases vascular permeability and upon histamine challenge → hypovolemic shock
- Endpoint:
  - Death
  - Decreased body temp (rectal or dermal)
Acellular pertussis (aP) vaccine safety test regulatory requirements

- Different published quantitative and qualitative methodologies described in regulations/guidelines:
  - Current edition European Pharmacopeia monograph 1356 for Adsorbed Pertussis Vaccine (Acellular, Component)
Japanese Requirements

1986 Minimum Requirements of Biological Products, Ministry of Health and Welfare, Japan regulations for Pertussis vaccines

- First acellular vaccines developed in Japan in 1981; accordingly the first HIST tests for acellular vaccines were also developed
- Transition from whole cell to acellular pertussis vaccine developed with average toxicities less than 1/10th of average whole cell vaccines (1)
- Whole cell pertussis HSD test not sensitive or accurate enough for control testing acellular vaccines
- A highly sensitive quantitative method for HIST activity in which rectal temperature change (decrease) is measured was developed by Ishida et.al. in 1979 (2); primarily used by Asian regulatory authorities
- Toxin reference included in assay (HSU)
- The regulation limit for HIST activity implemented in 1981; 0.8 HSU/mL and revised to 0.4 HSU/mL in 1991.

WHO Guidelines


- Final bulk vaccine lots should be tested for presence of active pertussis toxin using sufficiently sensitive histamine sensitization test (qualitative limit test)
- Reference toxin or positive control used in each test
- Acceptable limits based on consistency of manufacture approach
- Amount of active pertussis toxin in a new production lot should not exceed that present in lots shown to be safe in clinical trials
- TRS No.878 Annex 2 revised recently (draft)
  - Specific activity of reference standard or positive control should be calibrated in IU
  - “Development of an alternative to HIST is encouraged”
US FDA Regulations

- No specified HIST test in regulations – assays were established with CBER during licensure of acellular pertussis vaccines in the U.S.
- CBER approved assay is also a limit test for residual PTx activity that uses a lethal endpoint
- The test is designed to show that residual PTx activity in the vaccine is below an acceptable threshold
- Acceptable limits also based on consistency of manufacture approach
- Amount of active pertussis toxin in a new production lot should not exceed that present in lots shown to be safe in clinical trials
EU Regulations

Current edition Adsorbed Pertussis Vaccine (Acellular, Component) European Pharmacopeia monograph 1356

Purified PT bulk material (pre-adsorbed)
- Permits use of HIST test or CHO cell assay

Final Bulk Vaccine
- The EP HIST method is based on using a lethal end point
  - Final Lot of vaccine, twice the single human dose is injected
  - Two milligrams of histamine base are used for the challenge
  - Acceptance criteria: 0% deaths first test; NMT 5% deaths original and re-test combined
  - Sensitivity of the mouse strain is periodically assessed
Methods of Histamine Sensitization Testing used at Sanofi Pasteur in Canada

- Histamine Sensitivity Factor (HSF) – Canada
- Histamine Sensitization Assay (HSA) – US
- Absence of Residual Pertussis Toxin (Modified)
- Relative Toxicity
## Methods of Histamine Sensitization Testing used at Sanofi Pasteur in Canada (Part 1)

<table>
<thead>
<tr>
<th>Source of Mice</th>
<th>Number of Mice per group</th>
<th>Weight Range</th>
<th>Vaccine Dose (IP)</th>
<th>Challenge Histamine</th>
<th>Challenge /Vol/Route</th>
<th>Challenge (after Immunization)</th>
<th>Observation Period (post challenge)</th>
<th>Negative Control</th>
<th>Positive Control - PTx</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Canada</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine Sensitivity Factor (HSF)</td>
<td>16</td>
<td>13 to 18 g (3g span)</td>
<td>1 HD (0.5 mL)</td>
<td>Histamine diphosphate</td>
<td>0.7 mg (0.2 mL) IP</td>
<td>5 days</td>
<td>24 hours</td>
<td>16 mice (PBS)</td>
<td>400 ng</td>
</tr>
<tr>
<td><strong>USA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Histamine Sensitization Assay (HSA)</td>
<td>20</td>
<td>18 to 21.9 g</td>
<td>1 HD (0.5 mL)</td>
<td>Histamine dihydrochloride</td>
<td>1 mg (0.5 mL) IP</td>
<td>5 days</td>
<td>24 hours</td>
<td>20 mice (gPBS)</td>
<td>62.5 ng</td>
</tr>
<tr>
<td><strong>Ph. Eur.</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Absence of Residual Pertussis Toxin and Irreversibility</td>
<td>10</td>
<td>18 to 26g (4g span)</td>
<td>2 HD (1 mL)</td>
<td>Histamine dihydrochloride</td>
<td>2 mg (0.5 mL) IP</td>
<td>5 days</td>
<td>24 hours</td>
<td>10 mice (gPBS)</td>
<td>5.6, 16.7, 50, 150 ng</td>
</tr>
</tbody>
</table>

**Source:** NIH, CFW, NIH

**Weight Range:**
- Canada: 13 to 18 g (3g span)
- USA: 18 to 21.9 g
- Ph. Eur.: 18 to 26g (4g span)

**Vaccine Dose (IP):**
- Canada: 1 HD (0.5 mL)
- USA: 1 HD (0.5 mL)
- Ph. Eur.: 2 HD (1 mL)

**Challenge Histamine:**
- Canada: Histamine diphosphate
- USA: Histamine dihydrochloride
- Ph. Eur.: Histamine dihydrochloride

**Challenge /Vol/Route:**
- Canada: 0.7 mg (0.2 mL) IP
- USA: 1 mg (0.5 mL) IP
- Ph. Eur.: 2 mg (0.5 mL) IP

**Challenge (after Immunization):**
- Canada: 5 days
- USA: 5 days
- Ph. Eur.: 5 days

**Observation Period (post challenge):**
- Canada: 24 hours
- USA: 24 hours
- Ph. Eur.: 24 hours

**Negative Control:**
- Canada: 16 mice (PBS)
- USA: 20 mice (gPBS)
- Ph. Eur.: 10 mice (gPBS)

**Positive Control - PTx:**
- Canada: 400 ng
- USA: 62.5 ng
- Ph. Eur.: 5.6, 16.7, 50, 150 ng
Methods of Histamine Sensitization Testing used at sanofi pasteur in Canada (Part 2)

<table>
<thead>
<tr>
<th></th>
<th><strong>Canada</strong></th>
<th><strong>USA</strong></th>
<th><strong>Ph. Eur.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Histamine Sensitivity Factor</td>
<td>Histamine Sensitization</td>
<td>Absence of Residual Pertussis</td>
</tr>
<tr>
<td></td>
<td>(HSF)</td>
<td>Assay (HSA)</td>
<td>Toxin and Irreversibility</td>
</tr>
<tr>
<td>Acceptance criteria</td>
<td>Original Test – NMT one death</td>
<td>Original Test – NMT 2 deaths</td>
<td>Original Test – No deaths</td>
</tr>
<tr>
<td></td>
<td>(6.25%)</td>
<td>in group of exactly 20 mice</td>
<td>Retest – NMT 5% deaths on</td>
</tr>
<tr>
<td></td>
<td>Retest – NMT 6.25% deaths on</td>
<td>Retest – NMT 2 deaths in</td>
<td>original + retest</td>
</tr>
<tr>
<td></td>
<td>original + retest</td>
<td>groups of exactly 20 mice in</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 independent tests</td>
<td></td>
</tr>
<tr>
<td>Validity criteria</td>
<td>Minimum of 16 mice at challenge</td>
<td>Exactly 20 mice are</td>
<td>Minimum of 5 mice at challenge</td>
</tr>
<tr>
<td></td>
<td>No more than 1/16 deaths on</td>
<td>challenged in each group.</td>
<td>No death in negative control</td>
</tr>
<tr>
<td></td>
<td>negative control</td>
<td>NLT 14 deaths in the positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>At least 7/16 (43.75%) death on</td>
<td>control group</td>
<td>30 – 90% mice sensitive to 50</td>
</tr>
<tr>
<td></td>
<td>positive control</td>
<td>NMT 2 deaths in negative</td>
<td>ng dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>control group.</td>
<td></td>
</tr>
</tbody>
</table>
## Methods of Histamine Sensitization Testing used at sanofi pasteur in Canada cnt’d (Part 1)

<table>
<thead>
<tr>
<th><strong>Ph. Eur. Absence of Residual PTx and Irreversibility (modified)</strong></th>
<th><strong>Relative Toxicity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Mice per group</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>Source of Mice</strong></td>
<td>NIH</td>
</tr>
<tr>
<td><strong>Weight Range</strong></td>
<td>18 to 26 g (4g span)</td>
</tr>
<tr>
<td><strong>Vaccine Dose (IP)</strong></td>
<td>1 HD (0.5mL)</td>
</tr>
<tr>
<td><strong>Challenge Histamine</strong></td>
<td>Histamine dihydrochloride</td>
</tr>
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<td>2 mg (0.5 mL) IP</td>
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<td><strong>Challenge (after Immunization)</strong></td>
<td>5 days</td>
</tr>
<tr>
<td><strong>Observation Period (post challenge)</strong></td>
<td>24 hours</td>
</tr>
<tr>
<td><strong>Negative Control</strong></td>
<td>10 mice (gPBS)</td>
</tr>
<tr>
<td><strong>Positive Control - PTx</strong></td>
<td>50ng</td>
</tr>
</tbody>
</table>
Other Methods of Histamine Sensitization Testing used at sanofi pasteur in Canada cnt’d (Part 2)

<table>
<thead>
<tr>
<th>Acceptance criteria</th>
<th>Ph. Eur. Absence of Residual PTx and Irreversibility (modified)</th>
<th>Relative Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original Test – No deaths Retest – NMT 6.25% deaths on original + retest</td>
<td>Original Test – No more deaths in test than reference Retest – No more deaths in test than reference on original + retest</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Validity criteria</th>
<th>Minimum of 8 mice at challenge</th>
<th>Minimum of 8 mice/ group at challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No death in negative control</td>
<td>No deaths in negative control</td>
</tr>
<tr>
<td></td>
<td>30 – 90% control mice sensitive to 50ng dose</td>
<td>30 – 90% control mice sensitive to 50ng dose</td>
</tr>
</tbody>
</table>
Replacement of HIST Assay

- **The in vivo HIST assay is problematic**
  - Animal ethical concerns - large numbers of animals are used for this test
  - Inherent biological variability
  - Many variations in methodology
    - Different mice strains and weights, different doses of vaccine and histamine challenge, different histamine salts
    - Challenging for manufacturers
  - Cost

- **In vitro alternatives to HIST**
  - Highly desirable
  - Under active development internationally
Thank you