HISTAMINE SENSITIZATION TEST FOR ACELLULAR PERTUSSIS VACCINES

A review of acellular pertussis vaccine safety test regulatory requirements

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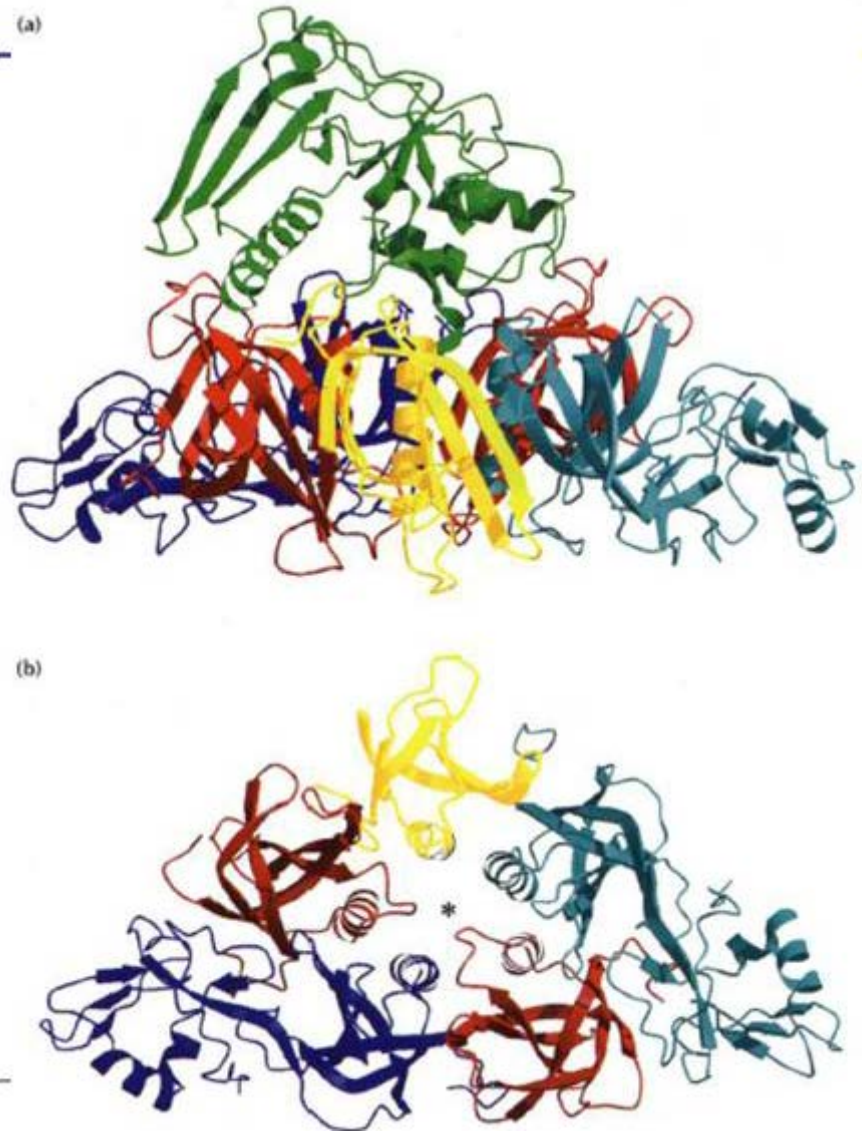
Pertussis Toxin: A member of the "AB_5" Holotoxin Family

A Protomer
(ADP-ribosyltransferase)

B Oligomer
(Binding)



Stein *et al.*, Structure 2 (1994): 45-57

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OVERVIEW

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)

OVERVIEW

Acellular pertussis (aP) vaccine safety test regulatory requirements

- Different published quantitative and qualitative methodologies described in regulations/guidelines:
  - **1986 Japanese Requirements for Pertussis Vaccines, The Minimum Requirements of Biological Products, Japan, Ministry of Health and Welfare, Japanese government publication**
  - **1998 WHO Technical Report Series, No. 878 Annex 2, Production and control of acellular pertussis vaccines**
  - **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.

Ref 1. Horiuchi Y, Takahashi M, Konda T, et al., 2001 Jpn. J. Infect. Dis., V 54 (5): 167–180

Ref 2. Ishida S, Kurokawa M, Asakawa S, Iwasa S. 1979. J Biol Stand., V 7(1):21-29

WHO Guidelines

1998 WHO issued guidelines for the production and control of acellular pertussis vaccines (monovalent and combined vaccines), WHO Technical Report Series, No. 878 Annex 2

- 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 – PH. Eur.
- Absence of Residual Pertussis Toxin (Modified)
- Relative Toxicity

Methods of Histamine Sensitization Testing used at sanofi pasteur in Canada (Part 1)

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

Methods of Histamine Sensitization Testing used at sanofi pasteur in Canada (Part 2)

	Canada **Histamine Sensitivity Factor (HSF)**	**USA** **Histamine Sensitization Assay (HSA)**	**Ph. Eur.** **Absence of Residual Pertussis Toxin and Irreversibility**
Acceptance criteria	Original Test – NMT one death (6.25%) Retest – NMT 6.25% deaths on original + retest	Original Test – NMT 2 deaths in group of exactly 20 mice Retest – NMT 2 deaths in groups of exactly 20 mice in 2 independent tests	Original Test – No deaths Retest – NMT 5% deaths on original + retest
Validity criteria	Minimum of 16 mice at challenge No more than 1/16 deaths on negative control At least 7/16 (43.75%) death on positive control	Exactly 20 mice are challenged in each group. NLT 14 deaths in the positive control group NMT 2 deaths in negative control group.	Minimum of 5 mice at challenge No death in negative control 30 – 90% mice sensitive to 50 ng dose

Methods of Histamine Sensitization Testing used at sanofi pasteur in Canada cnt'd (Part 1)

	Ph. Eur. **Absence of Residual PTx and Irreversibility (modified)**	**Relative Toxicity**
Number of Mice per group	10	10 each for test and reference
Source of Mice	NIH	NIH
Weight Range	18 to 26 g (4g span)	18 to 26 g (4g span)
Vaccine Dose (IP)	1 HD (0.5mL)	1 HD (0.5mL)
Challenge Histamine	Histamine dihydrochloride	Histamine dihydrochloride
Challenge /Vol/Route	2 mg (0.5 mL) IP	2 mg (0.5 mL) IP
Challenge (after Immunization)	5 days	5 days
Observation Period (post challenge)	24 hours	24 hours
Negative Control	10 mice (gPBS)	10 mice (gPBS)
Positive Control - PTx	50ng	4, 5.6, 16.7, 50, 150 ng

Other Methods of Histamine Sensitization Testing used at sanofi pasteur in Canada cnt'd (Part 2)

	Ph. Eur. **Absence of Residual PTx and Irreversibility (modified)**	**Relative Toxicity**
Acceptance criteria	Original Test – No deaths Retest – NMT 6.25% deaths on original + retest	Original Test – No more deaths in test than reference Retest – No more deaths in test than reference on original + retest
Validity criteria	Minimum of 8 mice at challenge No death in negative control 30 – 90% control mice sensitive to 50ng dose	Minimum of 8 mice/ group at challenge No deaths in negative control 30 – 90% control mice sensitive to 50ng dose

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