

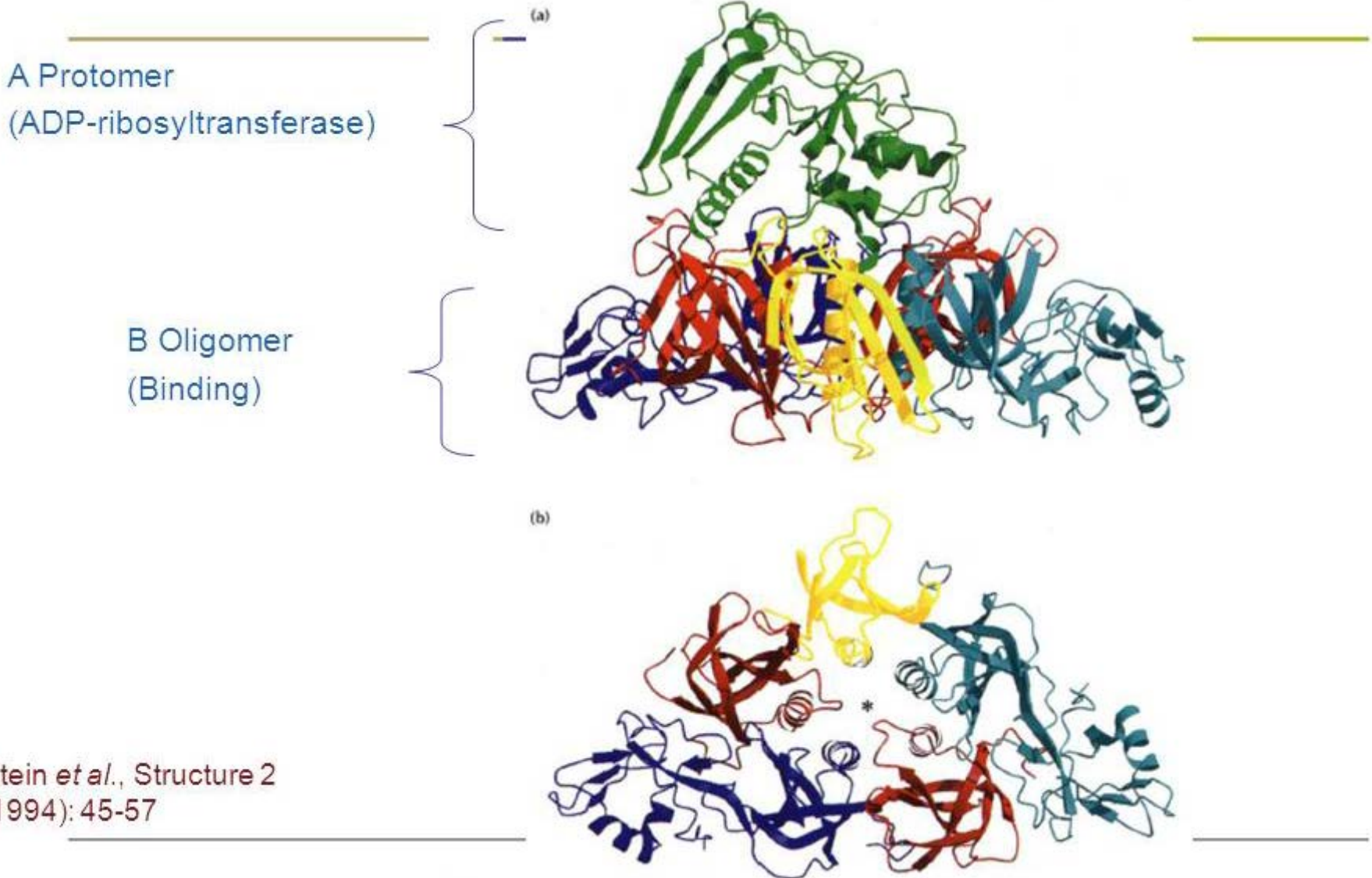
# HISTAMINE SENSITIZATION TEST FOR ACELLULAR PERTUSSIS VACCINES

A review of acellular pertussis vaccine safety test regulatory requirements

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# Pertussis Toxin: A member of the “AB<sub>5</sub>” Holotoxin Family



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

# OVERVIEW

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## 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

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## 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

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## 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

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## 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

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- **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

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## **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

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- 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)

	<b>Canada</b> <b>Histamine Sensitivity</b> <b>Factor</b> <b>(HSF)</b>	<b>USA</b> <b>Histamine Sensitization</b> <b>Assay</b> <b>(HSA)</b>	<b>Ph. Eur.</b> <b>Absence of Residual</b> <b>Pertussis Toxin and</b> <b>Irreversibility</b>
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)

	<b>Canada Histamine Sensitivity Factor (HSF)</b>	<b>USA Histamine Sensitization Assay (HSA)</b>	<b>Ph. Eur. Absence of Residual Pertussis Toxin and Irreversibility</b>
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)

	<b>Ph. Eur. Absence of Residual PTx and Irreversibility (modified)</b>	<b>Relative Toxicity</b>
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)

	<b><u>Ph. Eur.</u></b> <b>Absence of Residual PTx and Irreversibility (modified)</b>	<b><u>Relative Toxicity</u></b>
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

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- **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