27th November 2012

Highly Sensitive Histamine-Sensitization Test for Residual Activity of Pertussis Toxin In Acellular Pertussis Vaccine Using Body Temperature Monitoring

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Purpose of Histamine-Sensitization Test (HIST)

A.3 Production control

A.3.3 Control of pertussis antigen bulk materials

Residual activity of pertussis toxin. The amount of residual biologically active PT in the individually or co-purified antigens should be estimated after detoxification by means of a sufficiently sensitive test such as the HIST or the CHO-cell assay.

A.3.4 Control of final bulk

A.3.4.2.5 Residual activity of pertussis toxin

Each final bulk of vaccine should be tested for active PT using a HIST or another test that is sufficiently sensitive to detect the level of toxin activity agreed with the NRA.

Recommendations to Assure the Quality, Safety and Efficacy of Acellular Pertussis Vaccines (WHO 2011)

HIST method (Lethal endpoint)

Histamine shock in mice sensitized with Hemophilus pertussis vaccine was reported

(Parfentjevia and Goodline, J Pharmacol Exp Ther., 92(4): 411-3, 1948).

The assay method using lethality of mice as a response has widely and exclusively been adopted to determine the activity of PT in pertussis vaccine

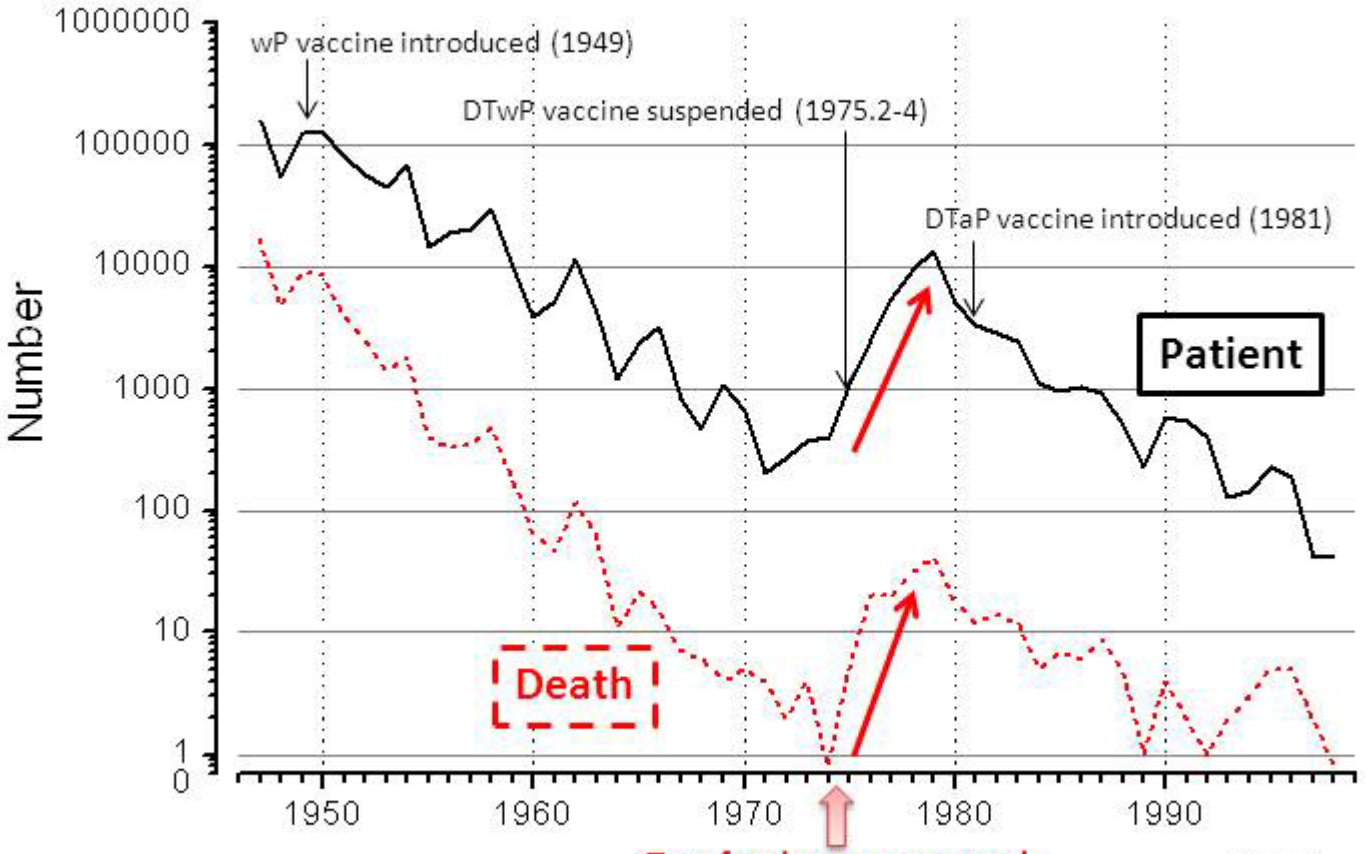
(Pittman, J Infect Dis. 89: 1951a, b).

Details of this method were provided in “Guidelines for the production and control of the acellular pertussis component of monovalent or combined vaccines.” (WHO TRS 878, Annex 2) in 1998.

Background

Why highly sensitive test method needed and developed in Japan?

Reported cases of Patient and Death of Whooping cough in Japan (1947-1998)



Data from the report of Ministry of Health and Welfare, Japan

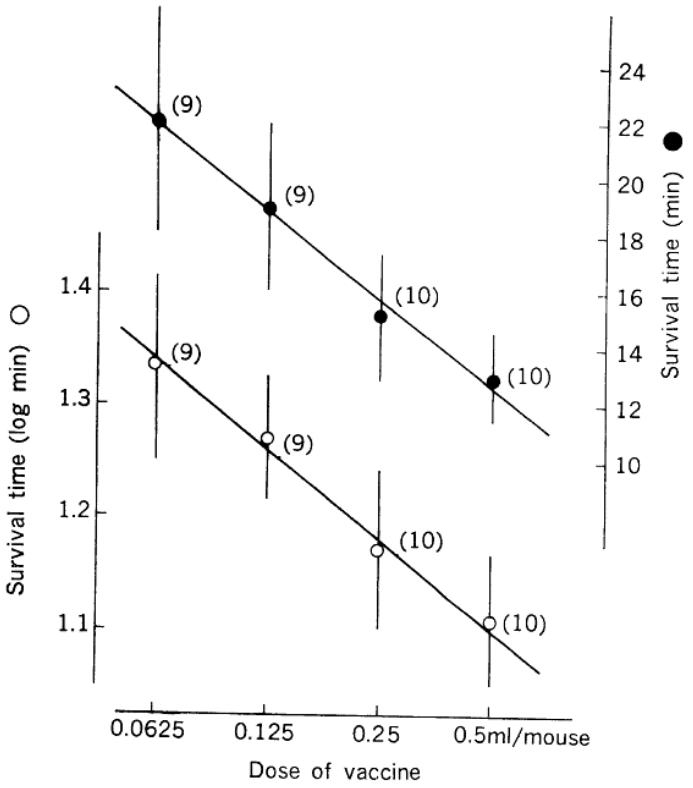
HIST method (Survival time)

Jpn J Med Sci Biol., 29: 139-150, 1976

A new biological assay method for his tamine-sensitizing factor using survival time as a response

Ishida S, Kurokawa M, Asakawa S

A new biological assay method using survival time of mice as a graded response after histamine challenge has been developed for the histamine sensitizing factor of pertussis bacilli. The method is relatively precise and reproducible in estimation and fairly sensitive in the validity tests of biological assay.



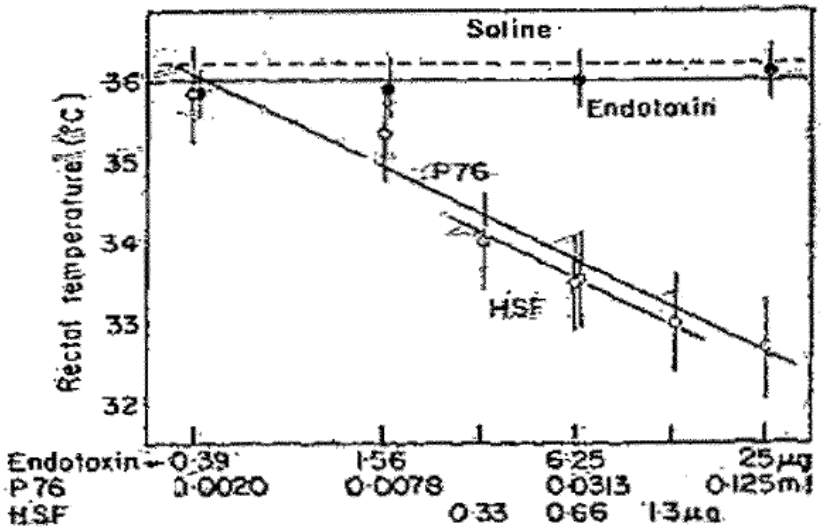
HIST method (Rectal temperature)

J Biol Stand., 7(1): 21-29, 1979

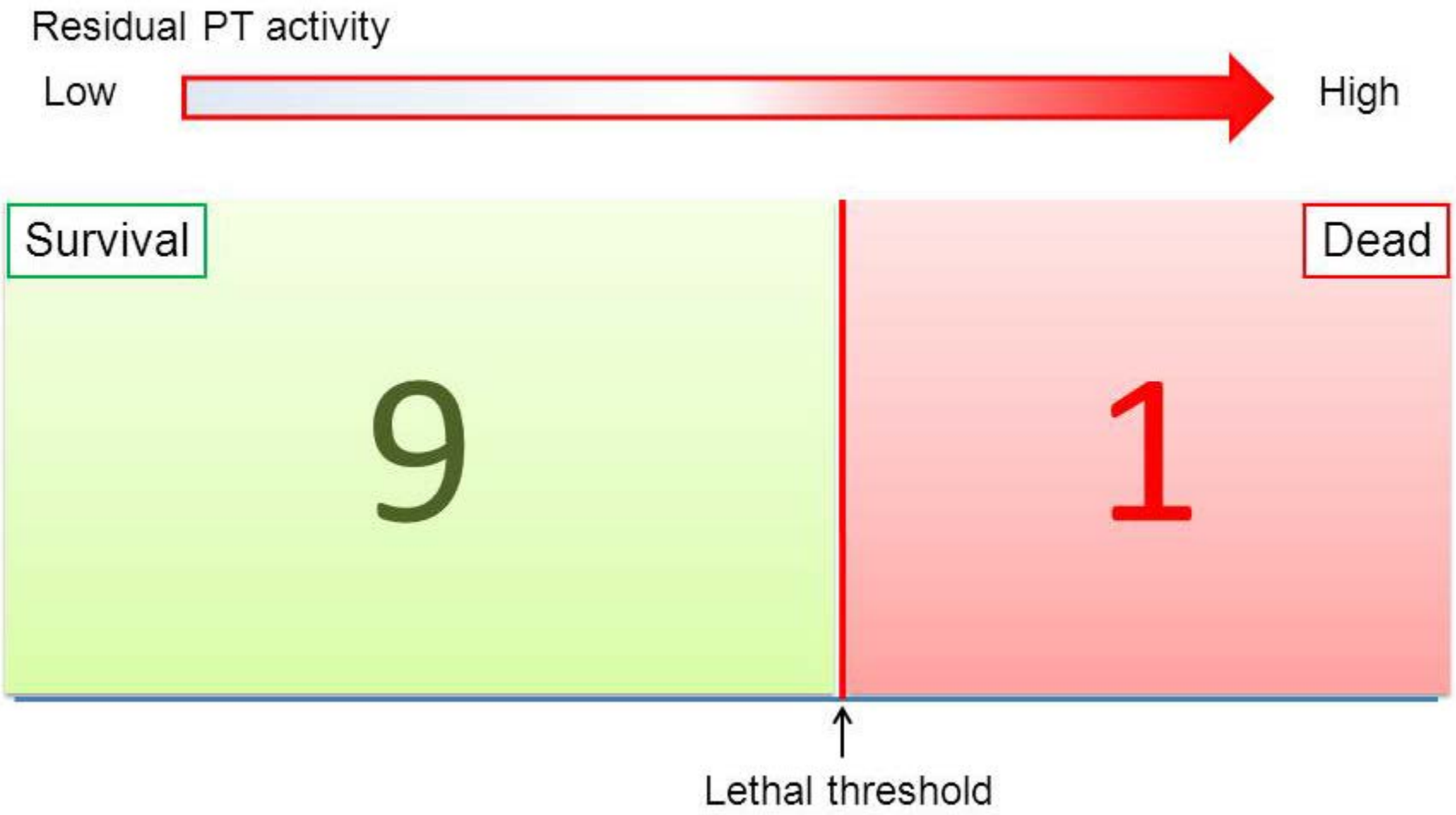
A sensitive assay method for the histamine-sensitizing factor using change in rectal temperature of mice after histamine challenge as a response

Ishida S, Kurokawa M, Asakawa S, Iwasa S

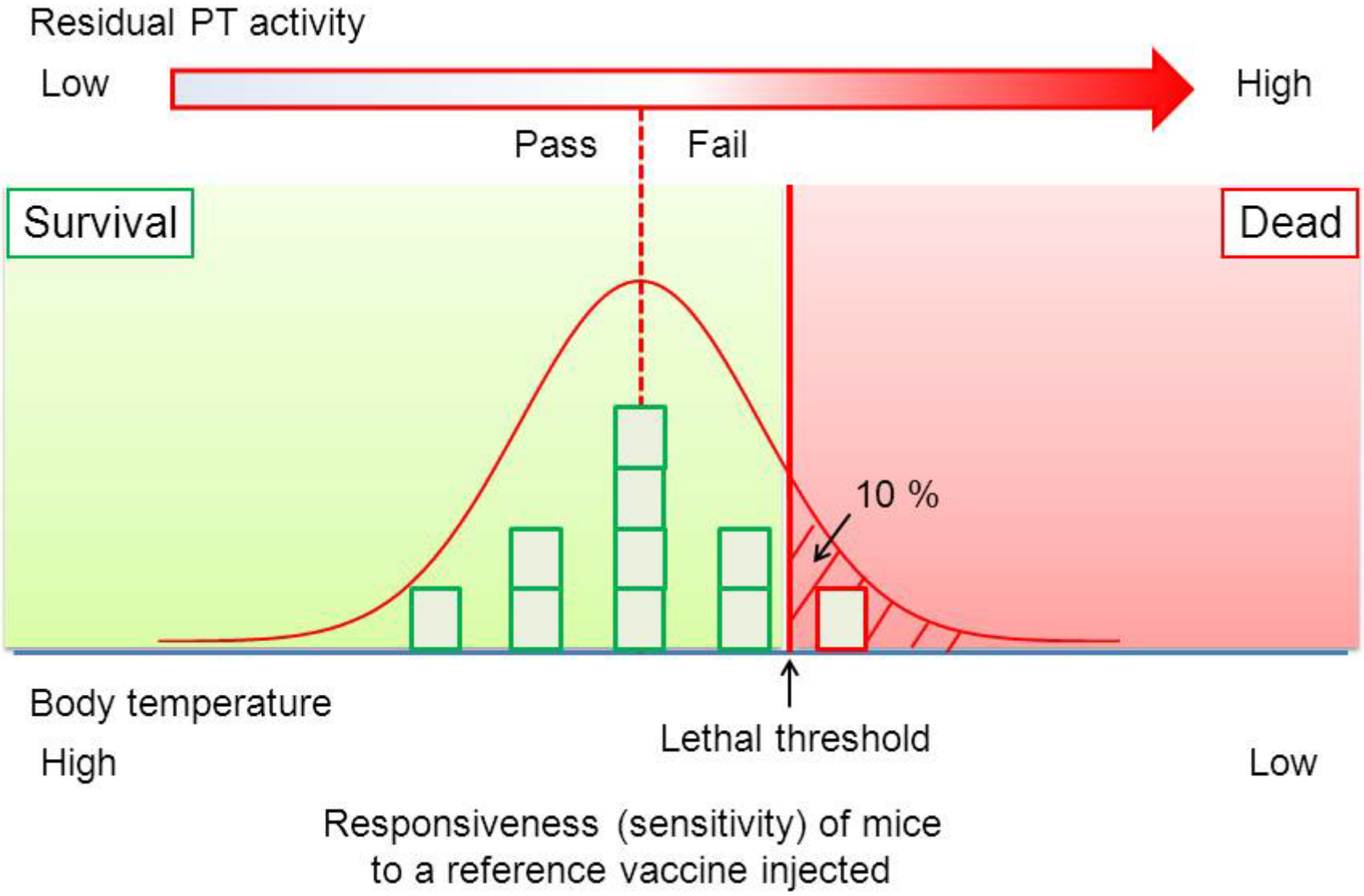
The sensitive and quantitative method for the histamine-sensitizing factor of pertussis vaccine using the rectal temperature fall in mice following histamine challenge has been developed. The method is much more sensitive than the methods that use the death rate or survival time of mice after histamine challenge and the precision and reproducibility of the method using rectal temperature are comparable to those of the method using survival time.



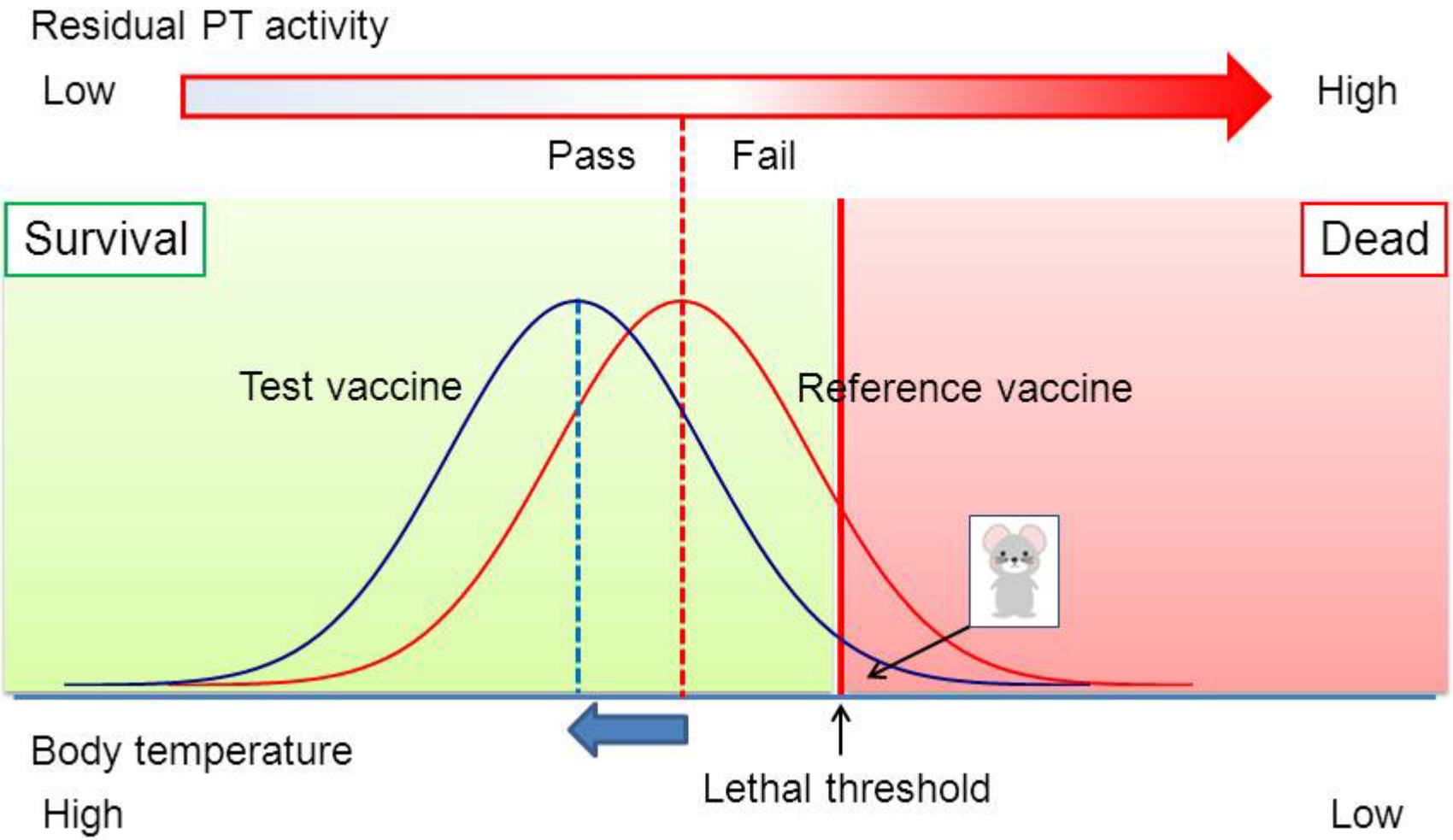
Lethal endpoint (quantal response)



Body temperature measurement (quantitative response)

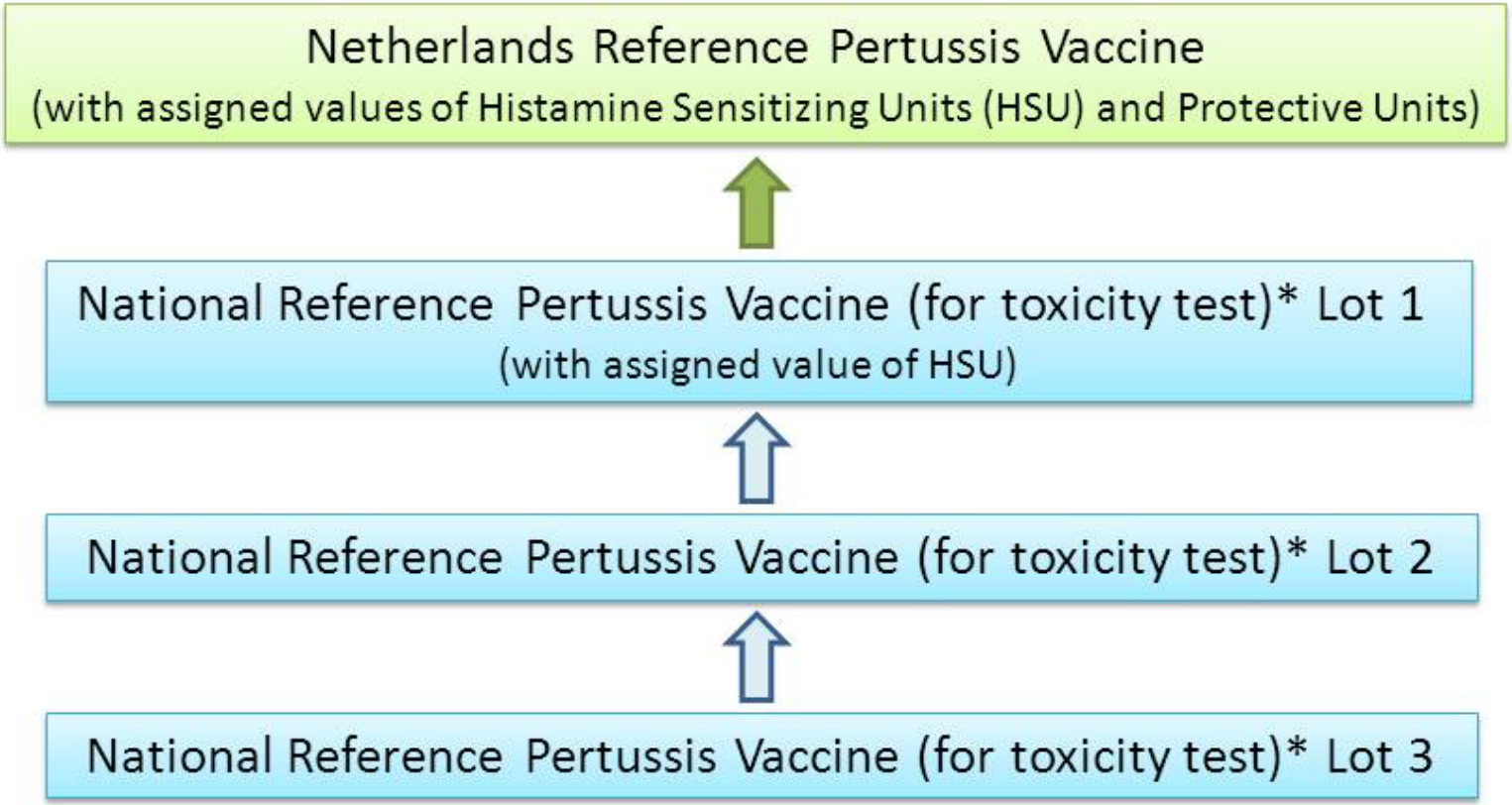


Body temperature measurement (quantitative response)



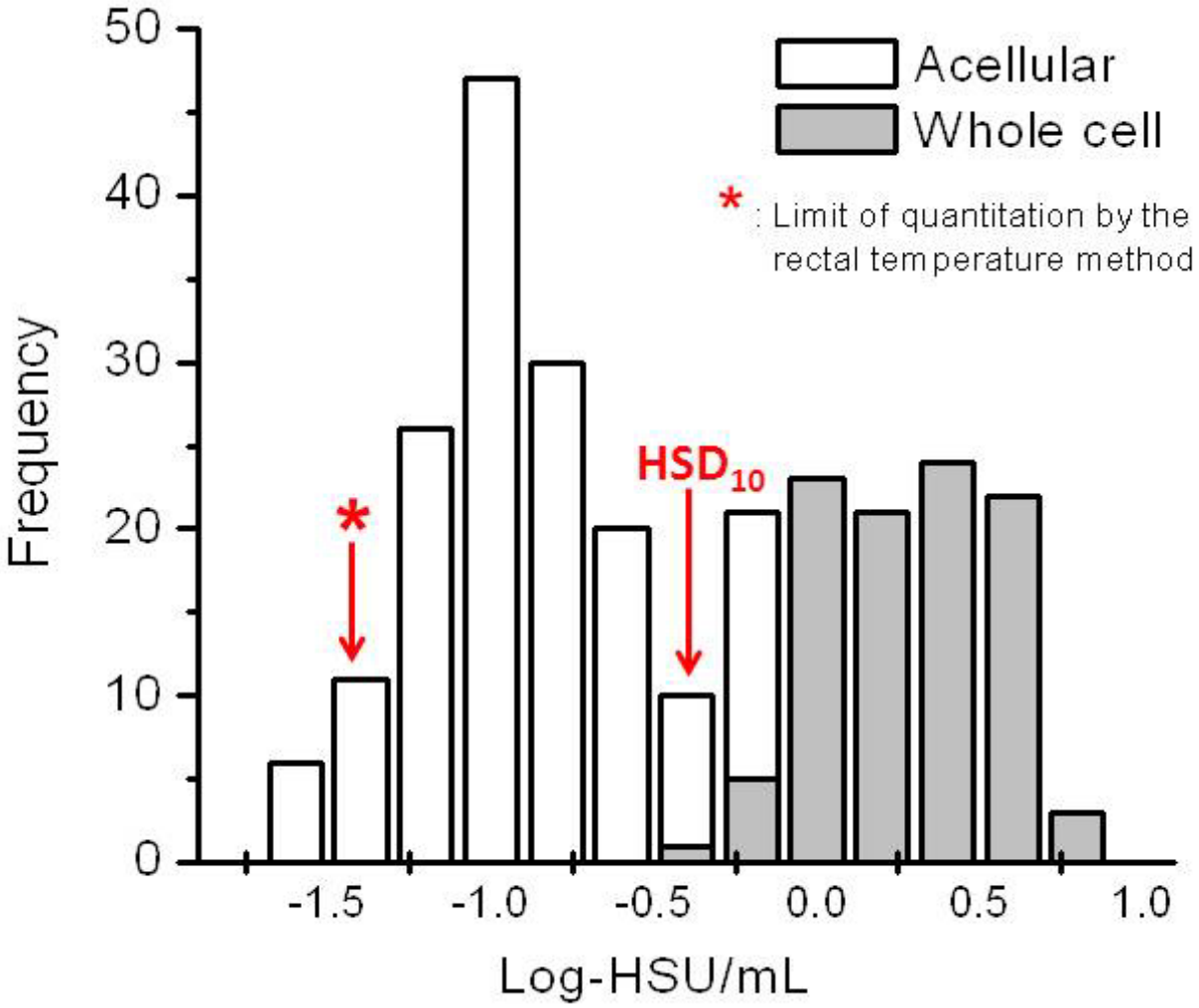
Use of reference preparation

Appropriate use of reference preparation is critically important for precise and reproducible estimation and comparison of the test results among different laboratories



* Lyophilized whole cell preparation of inactivated pertussis organisms

Residual activity of pertussis toxin in DTP vaccines including aP or wP (Japan)



Horiuchi *et al.*, Jpn J Infect Dis., 54(5): 167-80, 2001

Development of Dermal temperature HIST method

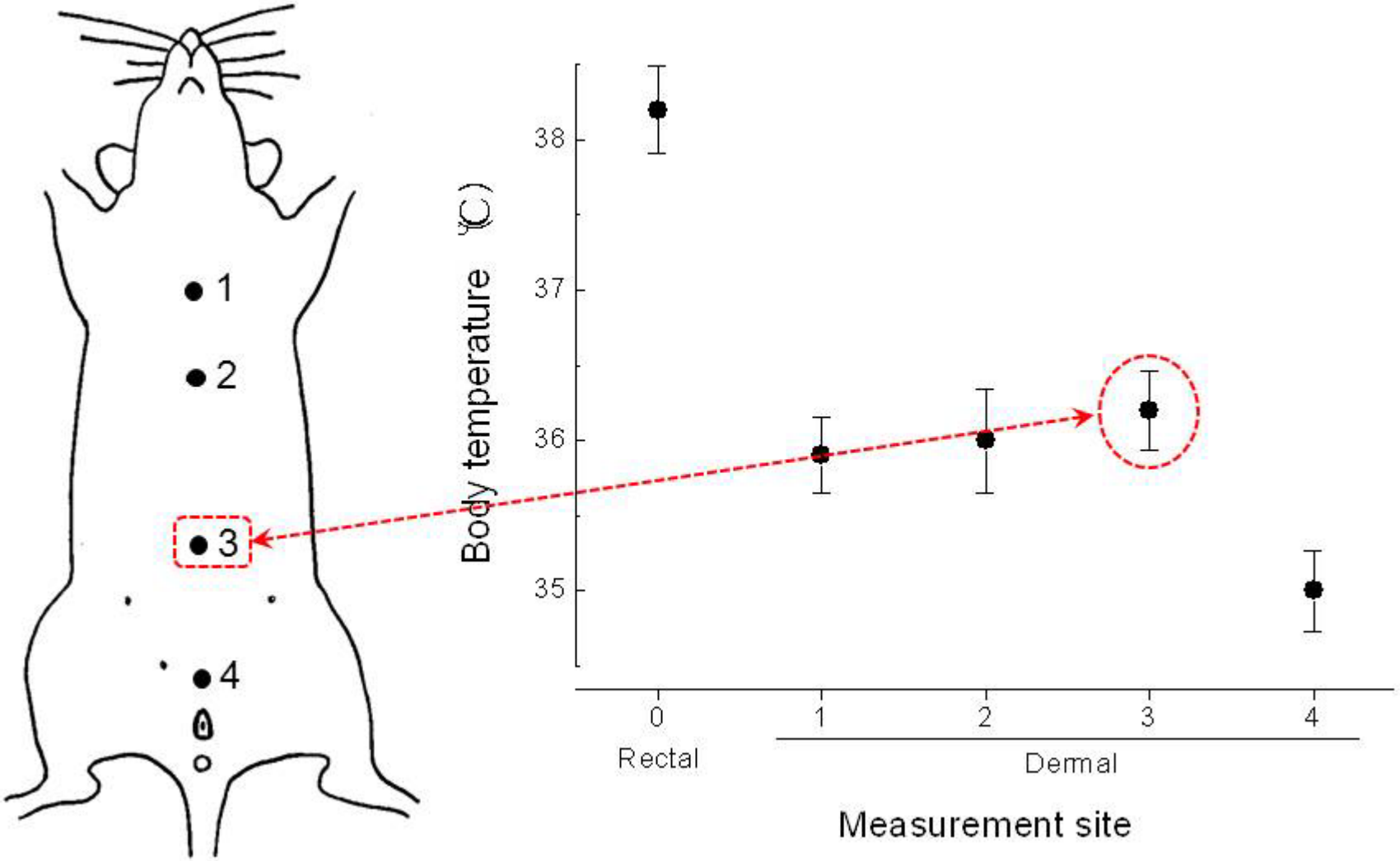
The HIST method using rectal temperature has the advantages in sensitivity and reproducibility to detect the residual PT activity in test vaccine and provides a quantitative estimate relative to the activity of the reference vaccine to allow comparison of the test results among different laboratories.

However, the HIST method using rectal temperature has been applied in only a few countries.

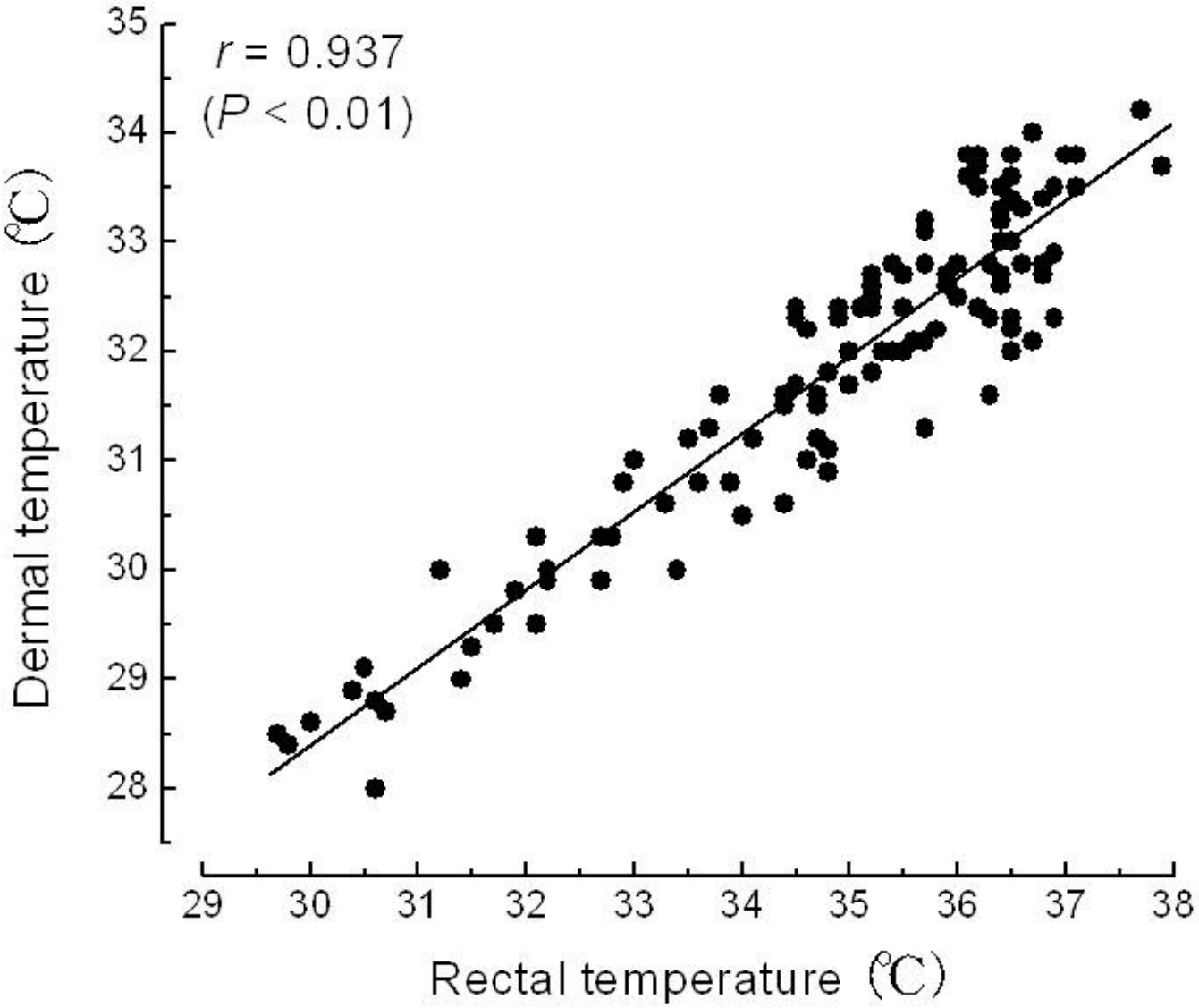
We examined the possibility of a quantitative HIST method using dermal temperature measured by infrared thermometer after histamine challenge for refinement of the test method to minimize discomfort on animals.

Ochiai *et al.*, Biologicals, 35(4): 259-264, 2007

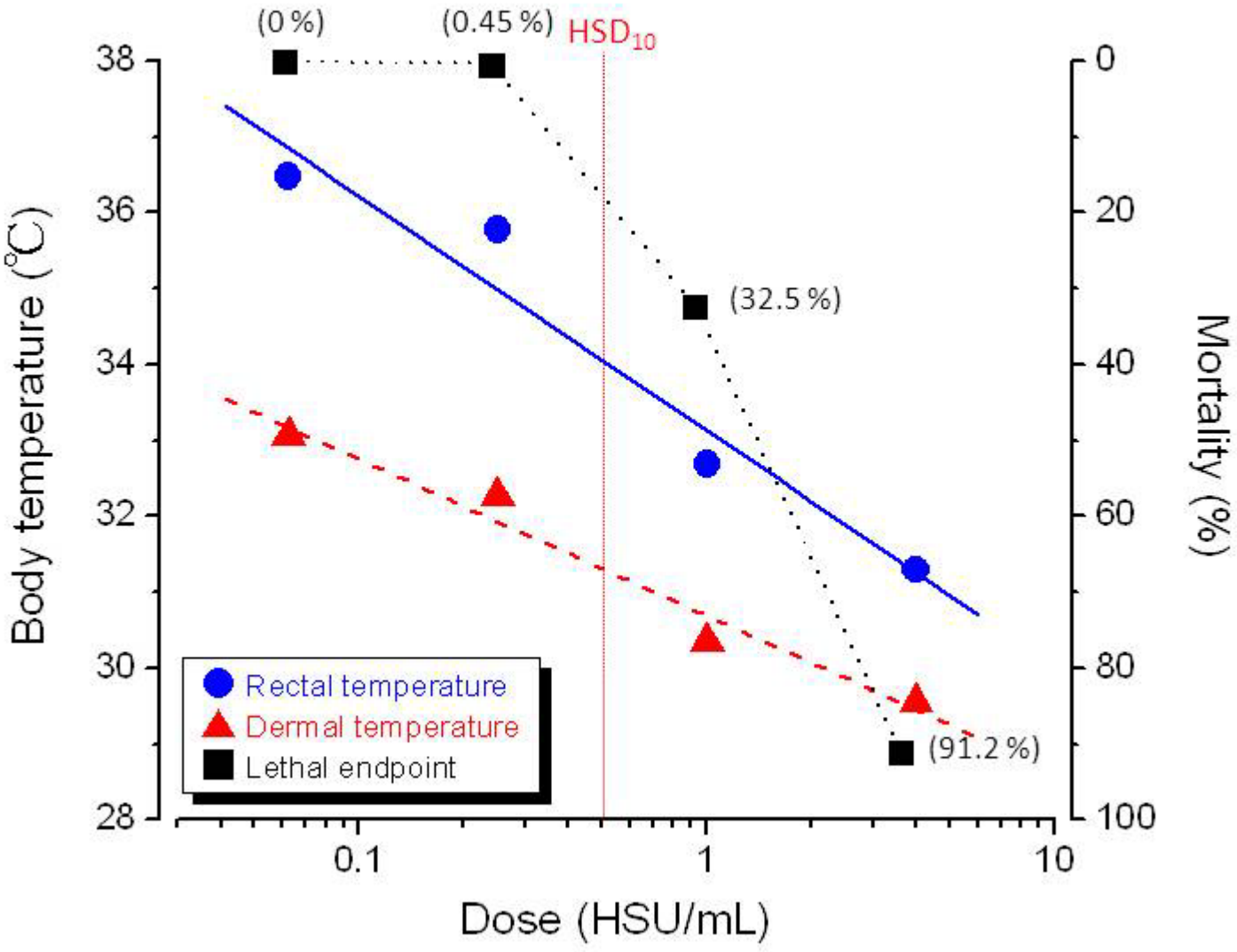
Body temperature of mice at different sites



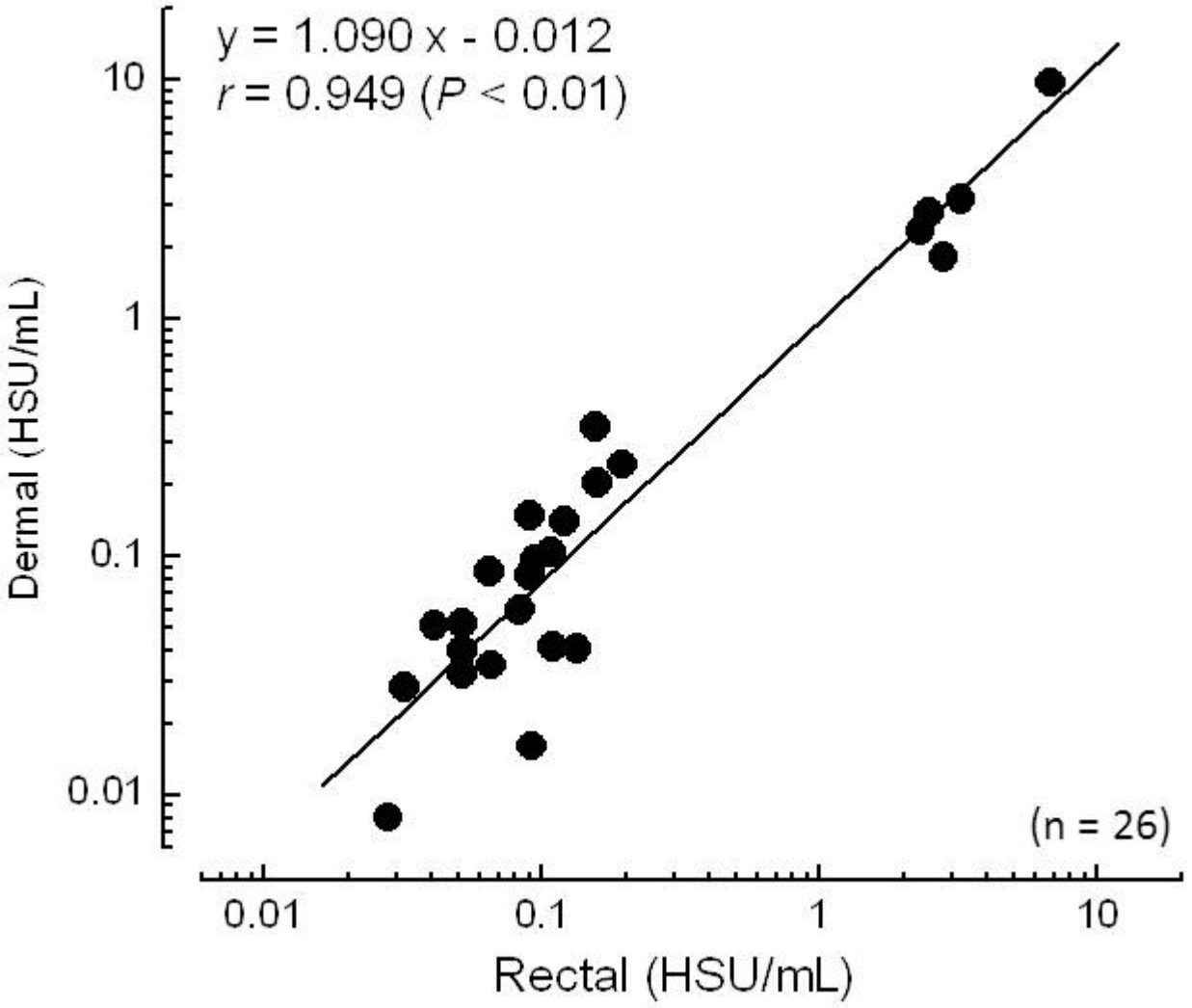
Correlation between dermal and rectal temperature of mice in the HIST



Dose-response lines of the reference vaccine in the HIST



Correlation between residual PT activities of DTaP vaccines by dermal and rectal temperature methods



Article for Dermal temperature HIST method

Hum Vaccin., 5(3): 166-171, 2009

Transferability of dermal temperature histamine sensitization test for estimation of pertussis toxin activity in vaccines.

Gaines-Das R, Ochiai M, Douglas-Bardsley A, Asokanathan C, Horiuchi Y, Rigsby P, Corbel MJ, Xing DK.

Biostatistics Department, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK.

We show that this method also can be used for more complex combination vaccines and is readily transferable. Furthermore use of dermal temperature provides a more precise quantitative estimate of toxin activity than the binary response, leading to an increase in information from a specified number of animals, or allowing a reduction in the number of animals required. We suggest that, pending the development of an alternative in vitro replacement method, the temperature based method may serve as an intermediate solution to the estimation of PT activity giving a precise estimate with reduction in animal numbers.

Article for Dermal temperature HIST method

Biologicals, 40(1): 36-40, 2012

Specificity and detection limit of a dermal temperature histamine sensitization test for absence of residual pertussis toxin in vaccines.

Jensen SE, Illigen KE, Badsberg JH, Hasløv KR.

Quality Control Dept, Statens Serum Institut, 5 Ørestads Boulevard, DK-2300 Copenhagen S, Denmark.

The acellular pertussis containing vaccine did not interfere with the pertussis toxin-induced temperature response recorded. In tests for presence of pertussis toxin in the pertussis vaccine preparation, the detection limit of the assay was estimated to approximately 5 ng pertussis toxin per human dose of pertussis toxoid. The dermal temperature-based assay was found to be a valid method to be applied in routine quality control of vaccines.

The revised WHO written standard

- *Residual activity of pertussis toxin* -

Currently the HIST method used for the determination of residual bioactive PT in licensed acellular pertussis vaccines is based on the response to a histamine challenge of mice injected with the vaccine on test. Two possible test outcomes (end-points) are in use. One is based on the lethal effect of the histamine challenge dose and the other on the decrease in body temperature post-histamine challenge.

Body temperature HIST method including dermal temperature has been provided in the revised WHO document, "Recommendations to Assure the Quality, Safety and Efficacy of Acellular Pertussis Vaccines (WHO 2011)".

Details of the body temperature HIST methods are provided in Appendix 1.

Advantages of Body temperature HIST methods compared with lethal endpoint method

1. The body temperature methods is much reliable and reproducible requiring much less re-testing compared to the lethal endpoint method*
   - *Reduction*
2. The body temperature methods use body temperature reduction as an endpoint instead of lethal endpoint
   - *Refinement*
3. The body temperature methods are completed 30 minutes later after histamine challenge versus 24 hours required by the lethal endpoint method
   - *Refinement*

* It was reported that 11.4% and 12% repeat testing on HIST were performed by OMCLs and manufacturers, respectively. (Xing D *et al*., Pharmeur Bio Sci Notes., 2010)

WHO International collaborative study on validation of an *in vitro* assay system as an alternative to current histamine sensitization test for acellular pertussis vaccines

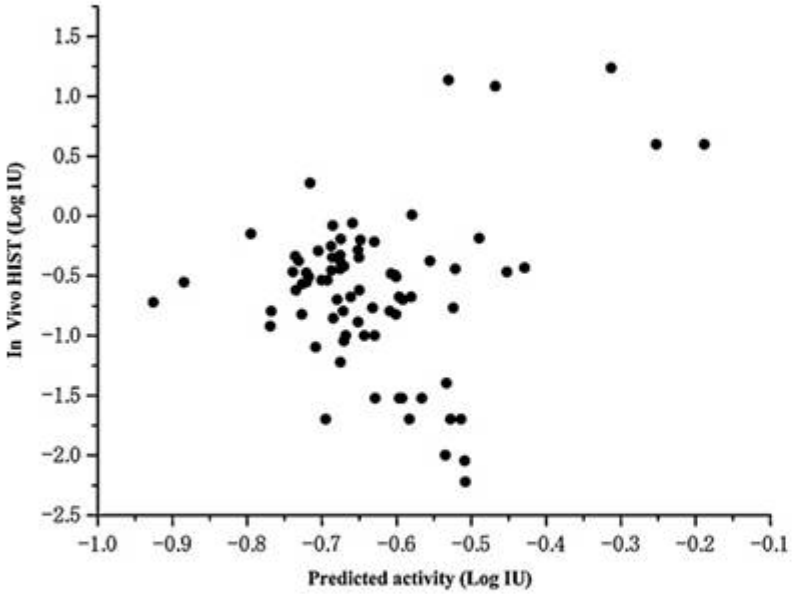
Geometric mean (GM) and 95% confidence limits (CL) of carbohydrate binding activity (BU), enzymatic activity (EU) and HIST activity (IU) for the three types of acellular pertussis based vaccine products included in the study.

		Activities		
		BU/dose	EU/dose	IU/dose
Sample 1, 6, 8	GM	0.49	0.27	0.24
	95% CL	(0.41–0.59)	(0.39–0.75)	(0.11–0.54)
Sample 2, 4, 7	GM	10.80	2.77	0.37
	95% CL	(5.57–20.93)	(2.39–12.83)	(0.13–1.09)
Sample 3, 5, 9	GM	0.10	0.12	0.20
	95% CL	(0.09–0.11)	(0.15–0.34)	(0.05–0.75)

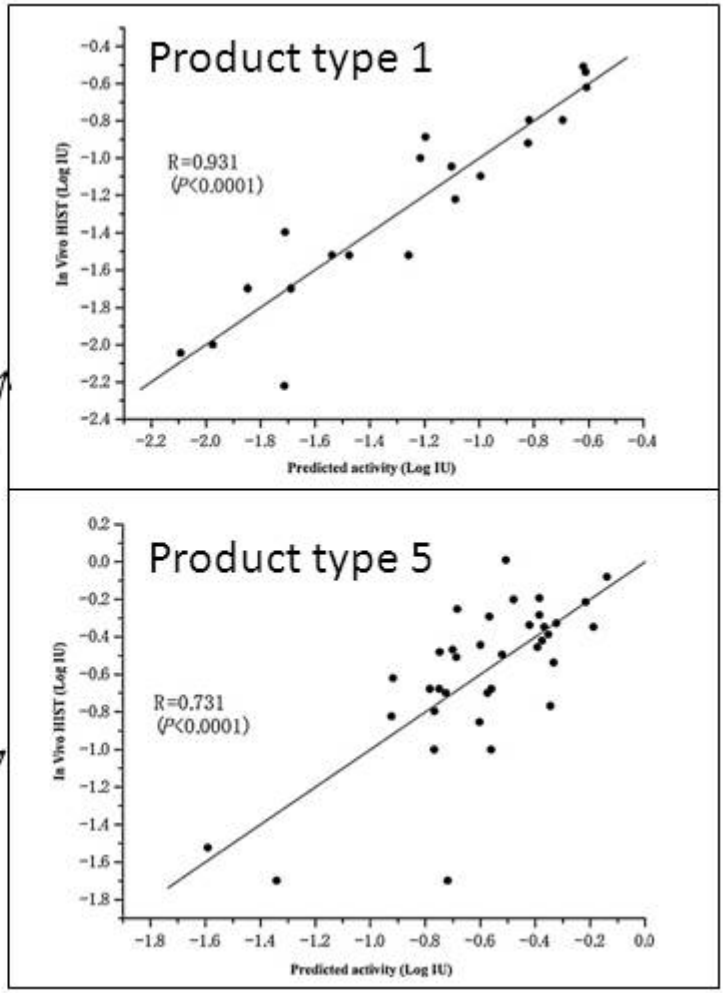
Xing D *et al.*, Biologicals, 40(6): 456-65, 2012

Relationship between PT activities predicted from the *in vitro* assays and measured by the temperature HIST method

Using common regression (b1, b2 and b3) and constant (C) factors



Using product specific regression and constant factors, respectively

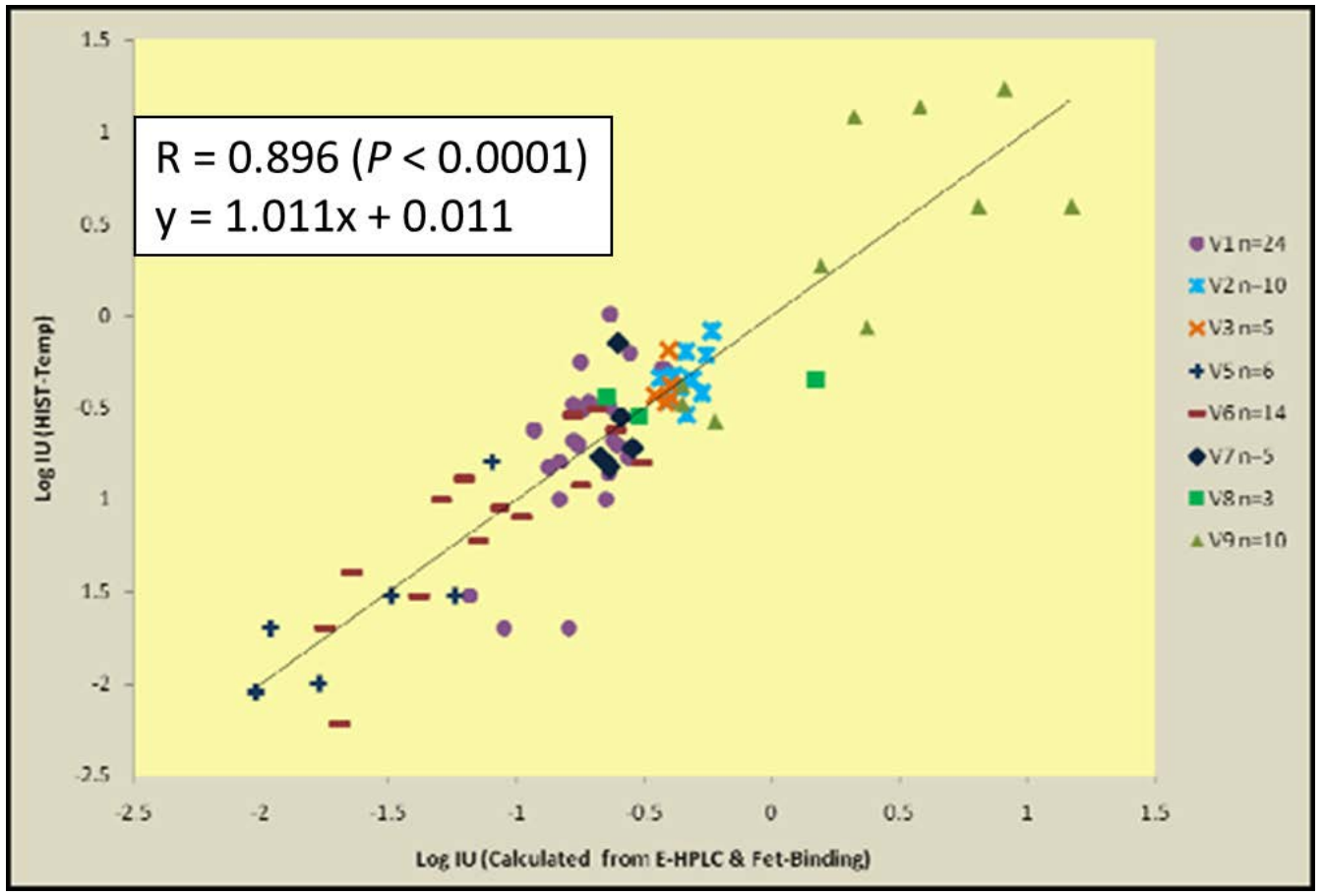


The predicted PT activities were calculated from data of the *in vitro* assays using a three-factors mathematical equation model.

log IU (predicted) = b1 x log Fet + b2 x log Fet x log Enz + b3 x log Enz + C

Yuen CT *et al.*, Vaccine, 28(21): 3714-3721, 2010

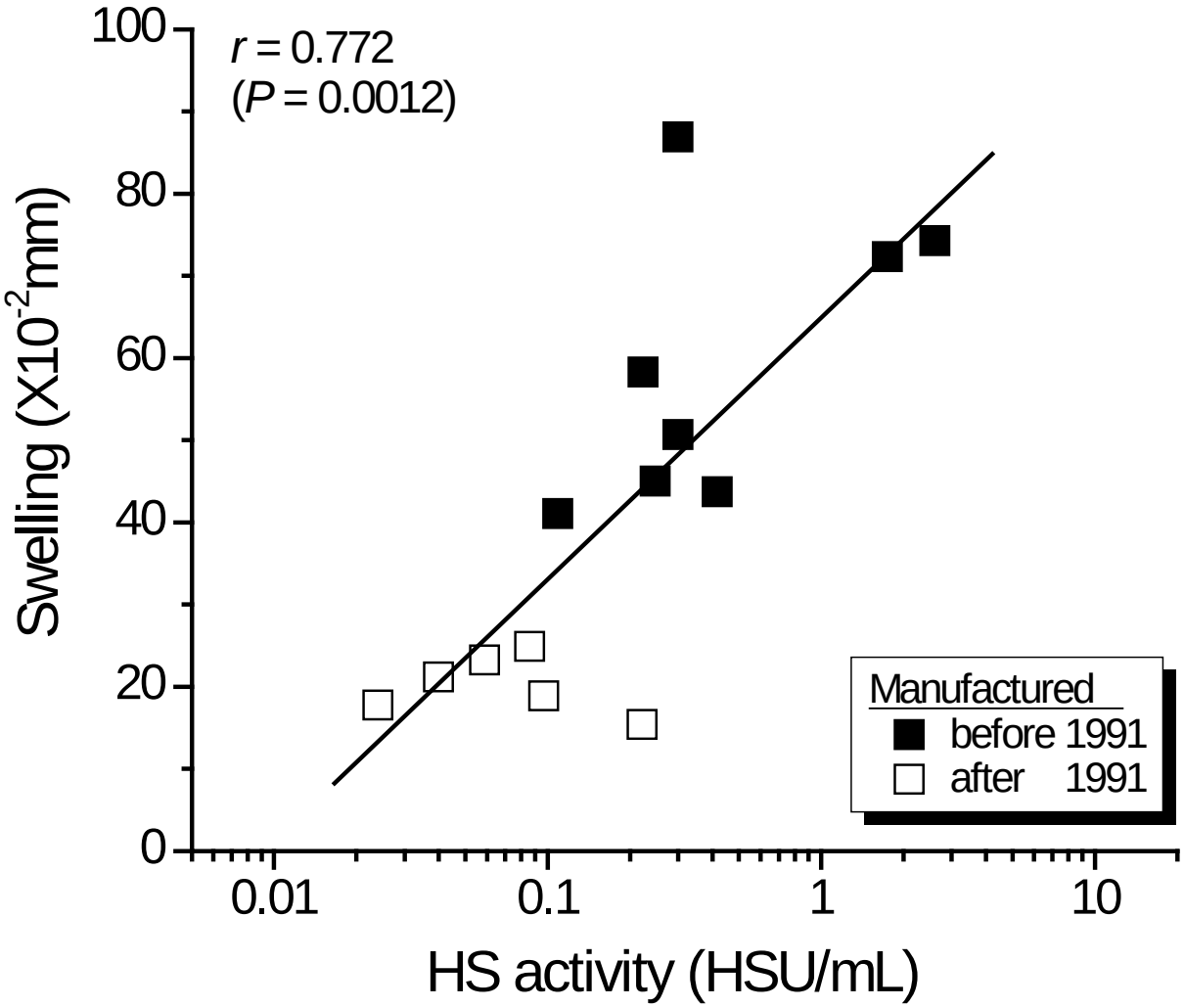
Relationship between PT activities predicted from the *in vitro* assays and measured by the temperature HIST method



The two factors mathematical equation model based on product specific regression and constant factors.

$$\log IU \text{ (predicted)} = b1 \times \log Fet + b2 \times \log Enz + C$$

Relationship between HS activity and local reactogenicity in mice



Yamamoto A *et al.*, Vaccine, 20(25-26): 3088-3094, 2002

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Thank you for your attention