Characterization of carbohydrate binding and ADP ribosyltransferase activities of chemically detoxified pertussis toxins

Hokyung, Oh. Ph.D.
National Center for Lot Release
NIFDS, KFDA, Republic of Korea.

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History

- International collaborative study on validation of an *in vitro* assay system as an alternative to current histamine sensitization test for acellular pertussis vaccines (2010 ~ 2011)

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Evaluation of an *in vitro* assay system as a potential alternative to current histamine sensitization test for acellular pertussis vaccines

Dorothy Xing [a,*], Chun-Ting Yuen [a], Catpagavalli Asokanathan [a], Peter Rigsby [a], Yoshinobu Horiuchi [b]

[a] National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar EN6 3QG, UK
[b] Division of Quality Assurance, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashimurayama-shi, Tokyo 208-0011, Japan

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ABSTRACT

The histamine sensitization test (HIST) is a lethal test for batch release of acellular pertussis or its combination vaccines (ACV). Large numbers of animals have been used and it is difficult to standardize. Therefore there is an urgent need to develop an *in vitro* alternative to HIST.

An *in vitro* test system has been developed as a potential alternative to HIST, to examine both the functional domains of PT based on a combination of enzyme coupled-HPLC (E-HPLC) and carbohydrate binding assays. We describe here an international collaborative study, which involved sixteen laboratories from 9 countries to assess the methodology transferability of the *in vitro* test system and its suitability for the testing of three different types of ACV products that are currently used worldwide. This study also evaluated further the relationship between the *in vivo* activity by HIST and the *in vitro* assay system.

The results showed that the methodology of the E-HPLC and carbohydrate binding assays are transferable between laboratories worldwide and is suitable for the three types of ACV products included in the study. Although direct correlation between the *in vitro* assay system and the *in vivo* HIST (temperature reduction assay) for each individual vaccine lot cannot be established due to the large variation in the HIST results, the observation that the mean estimates of the *in vitro* and *in vivo* activities gave the same rank order of the three vaccine types included in the study is encouraging. The *in vitro* systems provide reproducible product specific profiles which supports their use as a potential alternative to the HIST.

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History

- International collaborative study on validation of an in vitro assay system as an alternative to current histamine sensitization test for acellular pertussis vaccines (2010 ~ 2011)

Vaccine samples

Acellular pertussis containing vaccine (ACV) samples were kindly donated by GSK, Belgium, Sanofi Pasteur, Canada and Takeda Pharmaceuticals, Japan. They comprise three different types of ACV products currently in the worldwide market, with three batches of each vaccine type detoxified with glutaraldehyde and/or formaldehyde and they are:

- A purified acellular pertussis vaccine of five components detoxified with glutaraldehyde in combination with Diphtheria, tetanus, inactivated poliomyelitis and Haemophilus influenza type b conjugate vaccine

In vitro activities ↑

- A purified acellular pertussis vaccine of three components detoxified with glutaraldehyde and formaldehyde in combination with diphtheria and tetanus.
- A co-purified acellular pertussis vaccine detoxified with formaldehyde in combination with diphtheria and tetanus.

History

RESEARCH PAPER

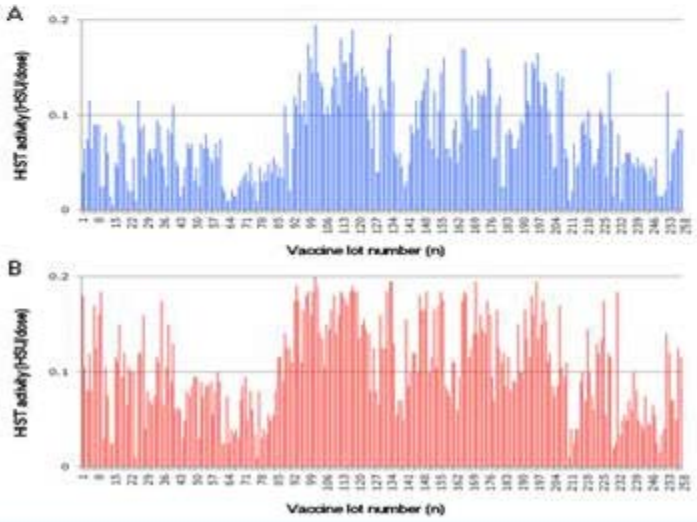
Retrospective analysis of the results of acellular pertussis vaccine toxicity tests performed in Korea

Hokyung Oh,[1,3] Jeewon Joung,[1] Byoungguk Kim,[1] Halim Lee,[1] Woosun Lee,[2] Seung-Hwa Hong[1,*] and Byung-Yoon Ahn[3,*]

[1]National Center for Lot Release, National Institute of Food & Drug Safety Evaluation, Korea Food & Drug Administration, Chungcheongbuk-do, South Korea; [2]International Trade & Statistic Office, Korea Food & Drug Administration, Osong, Chungcheongbuk-do, South Korea; [3]School of Life Sciences and Biotechnology, Korea University, Anam-dong, Sungbuk-gu, Seoul, South Korea

Key words: acellular pertussis vaccine, specific toxicity test, mouse body weight gain test, leukocytosis-promoting test, histamine sensitization test

Specific toxicity test is a major quality control test for acellular pertussis (aP) vaccines performed by manufacturers and regulatory authorities. The 'mouse body weight gain test (MWGT)', the 'leukocytosis-promoting test (LPT)' and the 'histamine sensitization test (HIST)' have been conducted to check the specific toxicity of all batches of aP vaccines used in Korea through the national quality control program, which requires a lot of animals, labor and time. In this study, test results obtained in the past 9 y from a total of 258 lots of aP vaccines were examined retrospectively to evaluate the three test methods. A pairwise comparison of the test results indicated a good correlation between LPT and HIST, whereas MWGT showed no correlation with either LPT or HIST. Moreover, the reversion to toxicity was higher than the residual toxicity in the majority of lots tested by HIST, which indicated that the histamine-sensitizing toxicity, although rated within a safe range, increased during the vaccine storage. Thus, the vaccine safety test results accumulated in the past might be useful for the improvement of test protocols.



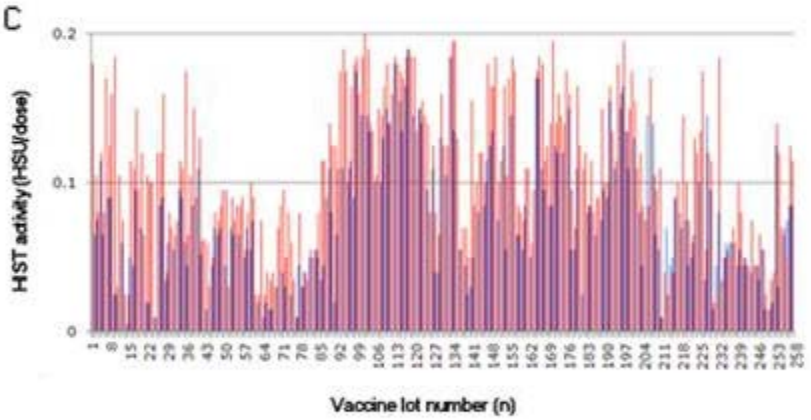


Figure 2. Comparison of the HIST results for residual toxicity and those for reversion to toxicity (n=258). (A) HIST results for residual toxicity. (B) HIST results for reversion to toxicity. (C) Overlaps of the two results.

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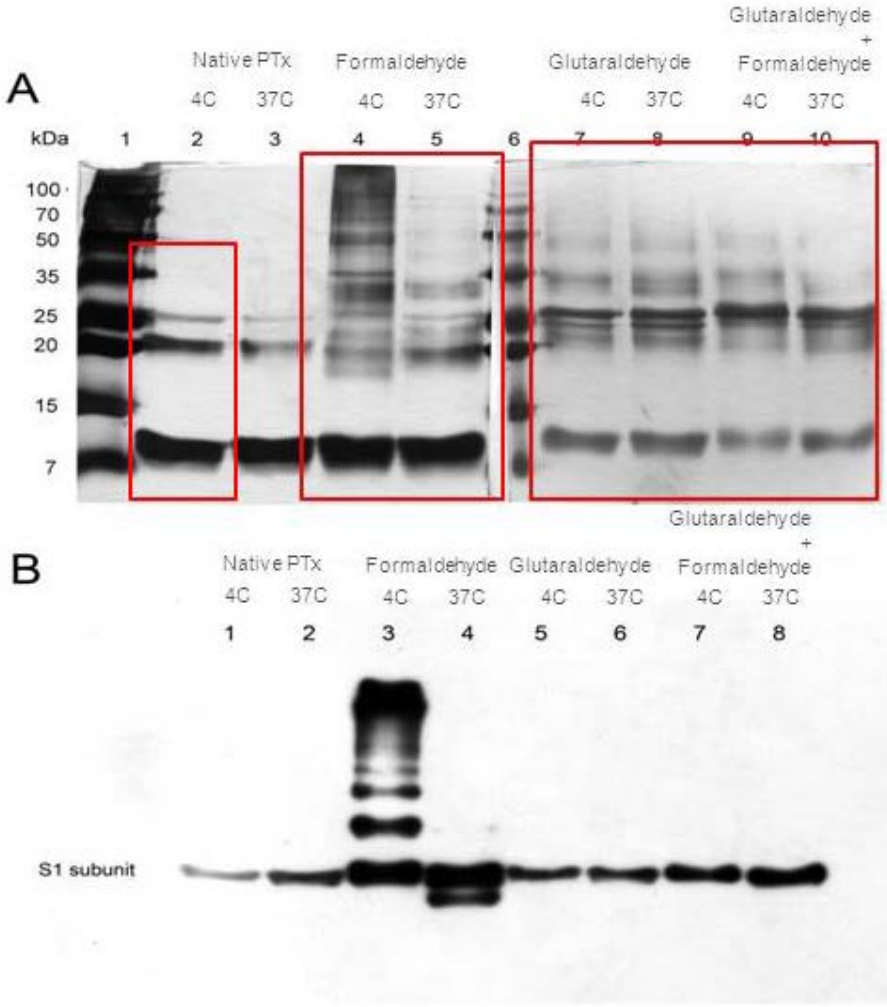
Outline of Study Design

- The aims of the study
  - is to characterize the *in vitro* biochemical assays according to detoxifying agents.
  - is to further evaluate the reversion to toxicity between the aP vaccines using the *in vitro* biochemical assays

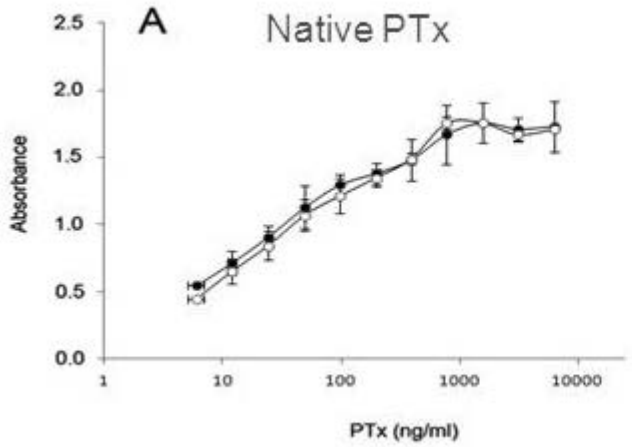
- Materials
  - detoxified PTx (PTx is purified from strain Tohama I) according to formaldehyde (at final concentration 0.26%), glutaraldehyde (at final concentration 0.05%), mixture of glutaraldehyde (0.05%) and formaldehyde (0.26%)

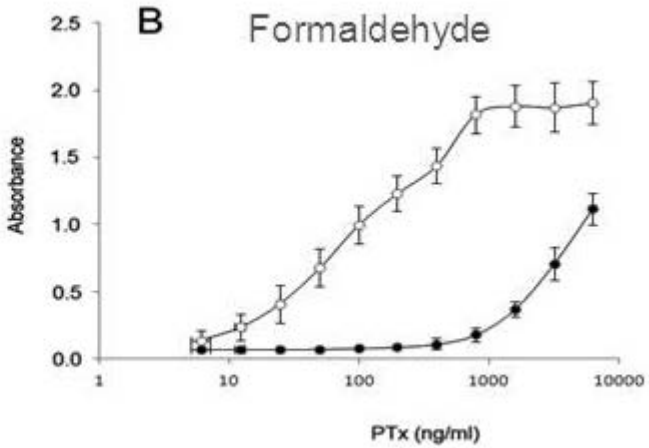
- Methods
  - Carbohydrate binding assay
  - Enzymatic HPLC assay
  - Silver staining and western blotting

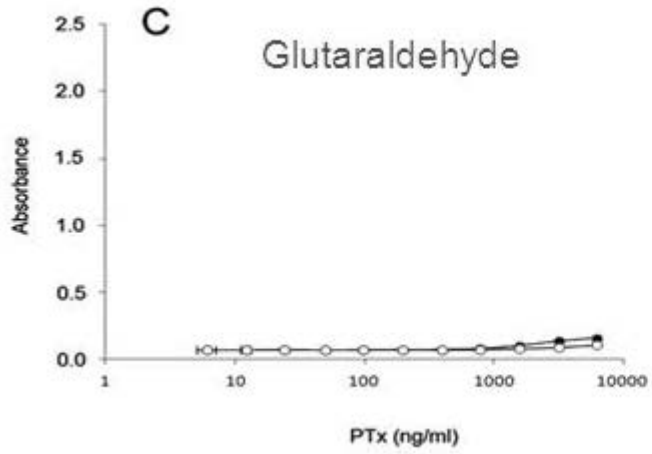
Cross-linking patterns of the chemically detoxified PTx

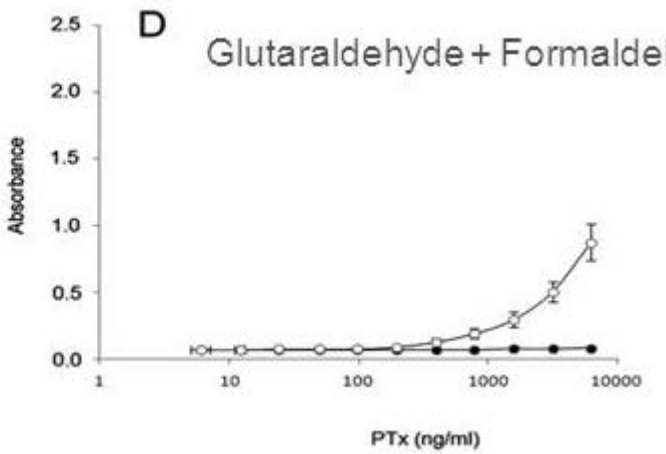


Carbohydrate binding activities according to detoxifying agents and storage conditions

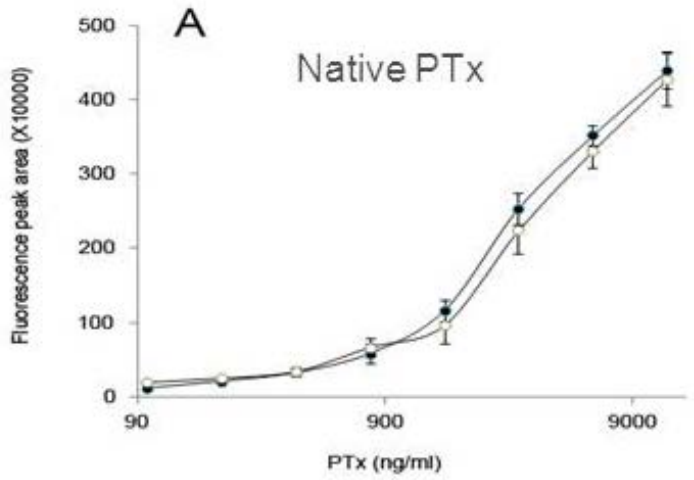


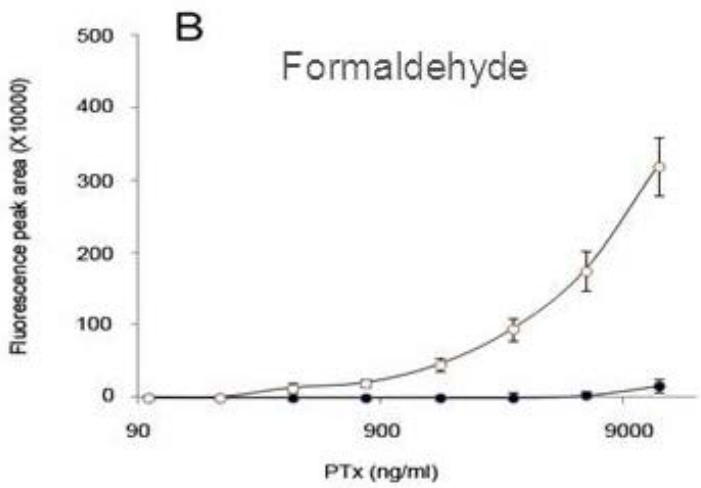


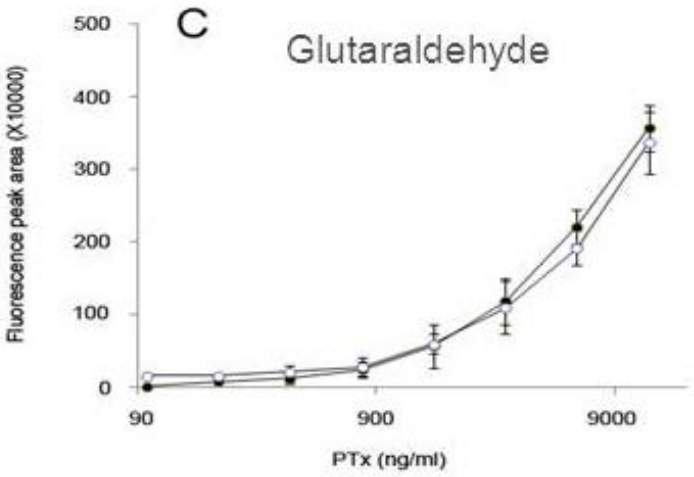


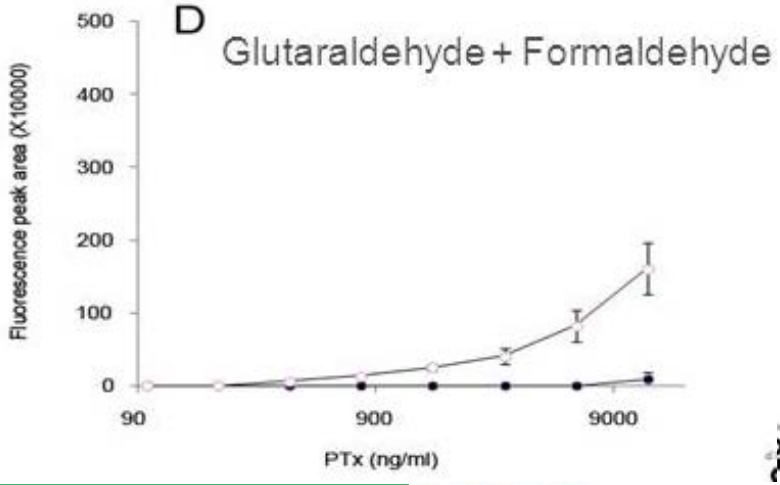


ADP-ribosyltransferase activities according to detoxifying agent and storage condition.

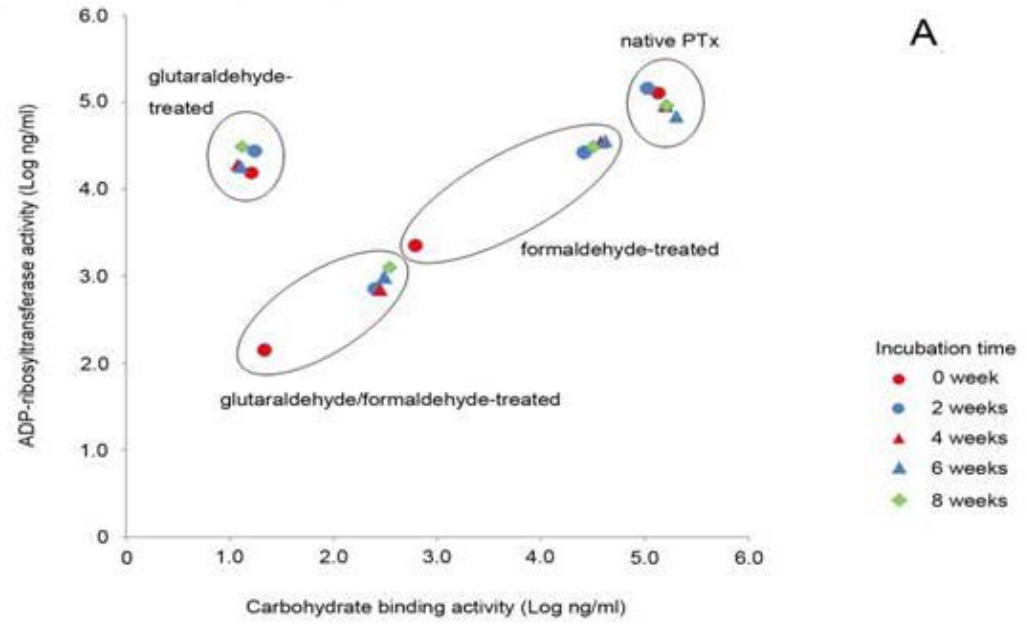


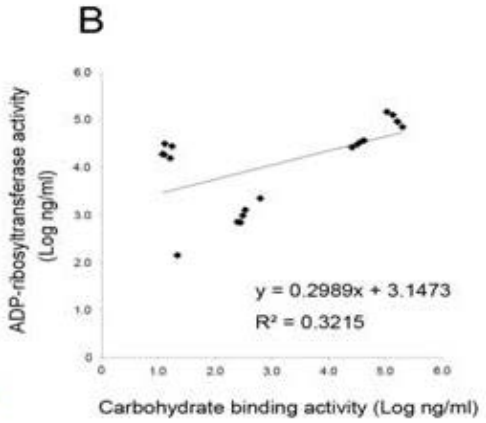


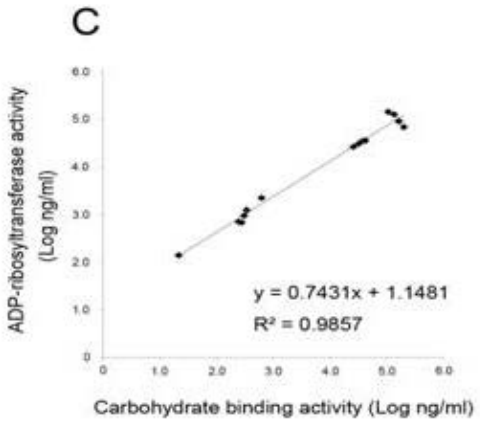




Relationship between the carbohydrate binding and ADP-ribosyltransferase activities according to detoxifying agents.







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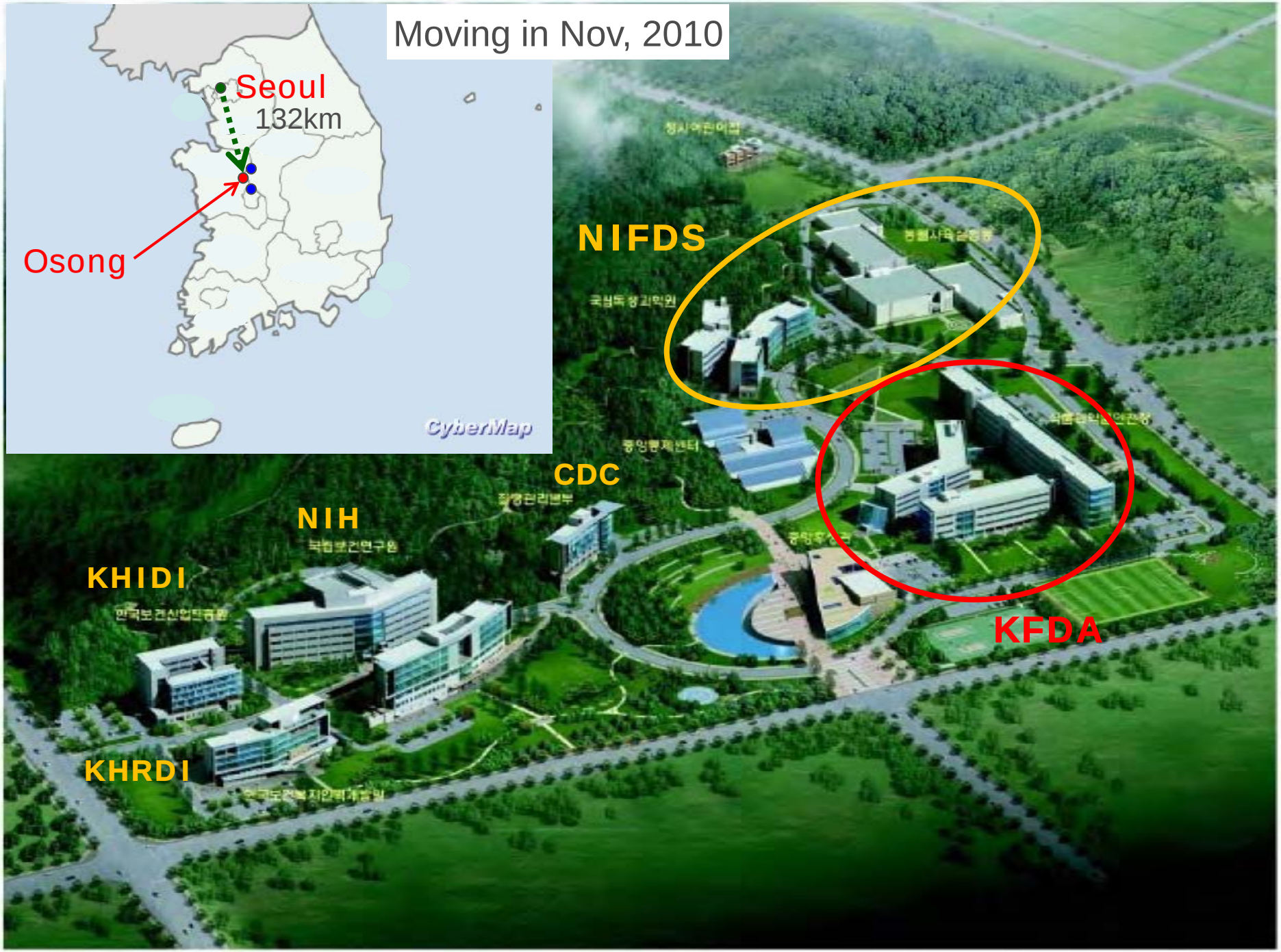
Summary

1. Formaldehyde treatment effectively detoxified the pertussis toxin, but its toxicity could be reverted during storage at 37℃.
2. Glutaraldehyde treatment did not inactivate ADP-ribosyltransferase activity, but it completely and irreversibly inactivated the carbohydrate binding activity of pertussis toxin.
3. Glutaraldehyde and formaldehyde treatment inactivated two *in vitro* biochemical activities and showed reversion to toxicity, although a much lesser extent than formaldehyde treated samples.
4. The reversion to toxicity was shown in just two weeks.
5. The *in vitro* biochemical assays may be approached as a vaccine specific manner rather than common methods used for all types of aP vaccines.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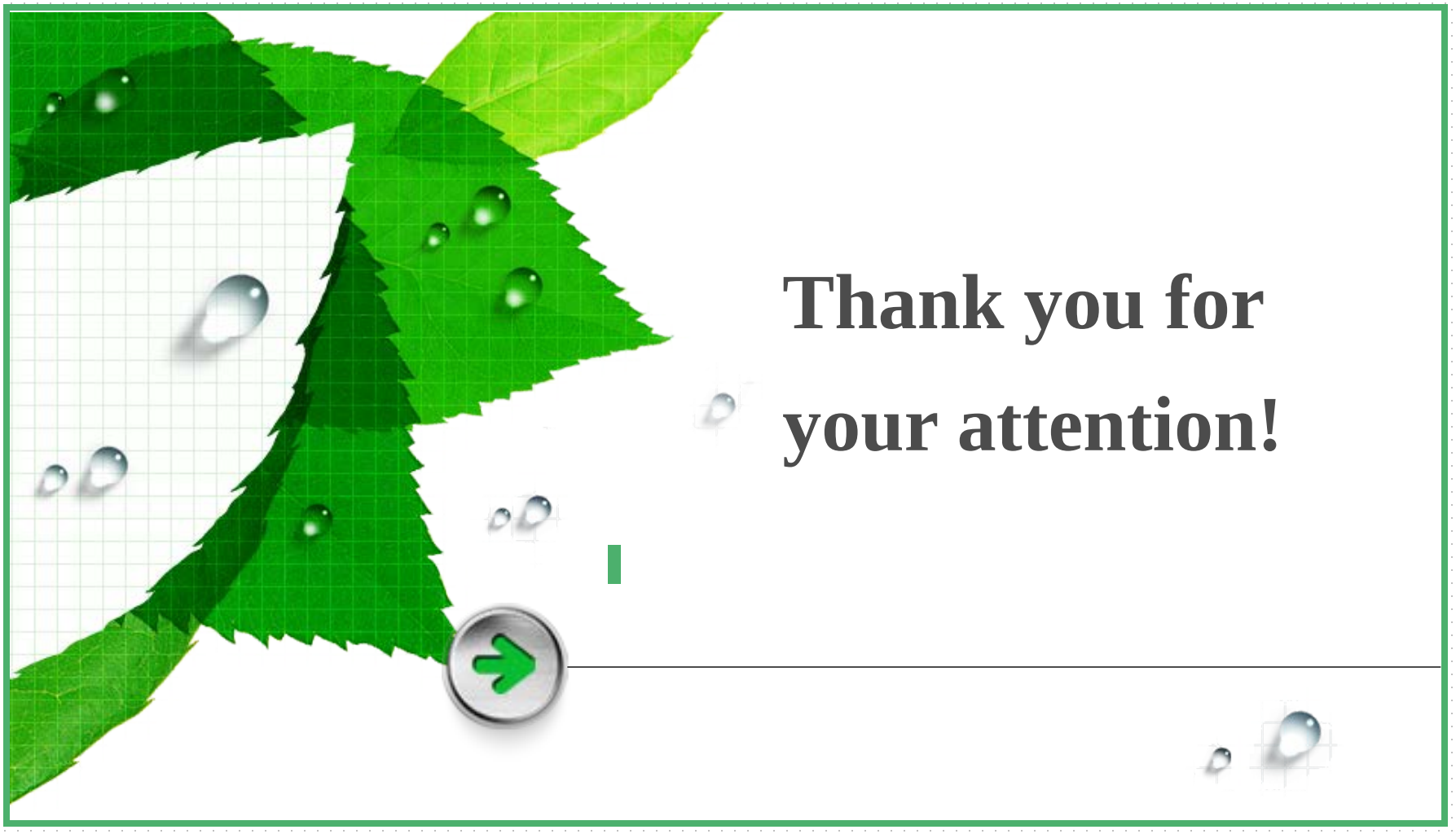
Seoul
132km
Osong
CyberMap
NIFDS
CDC
NIH
KHIDI
KHRDI
KFDA



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