

# International Workshop on Alternatives to HIST for Acellular Pertussis Vaccines (BSP114 Collaborative Study, 2012)

Analyses of pertussis toxin ADP-ribosyltransferase and  
carbohydrate-binding activities as an *in vitro* alternative  
to *in vivo* HIST

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National Institute for Biological Standards and Control  
Assuring the quality of biological medicines



# Introduction

## Regulatory toxicity test: *In vivo* HIST

*In vitro* human cell-based model:

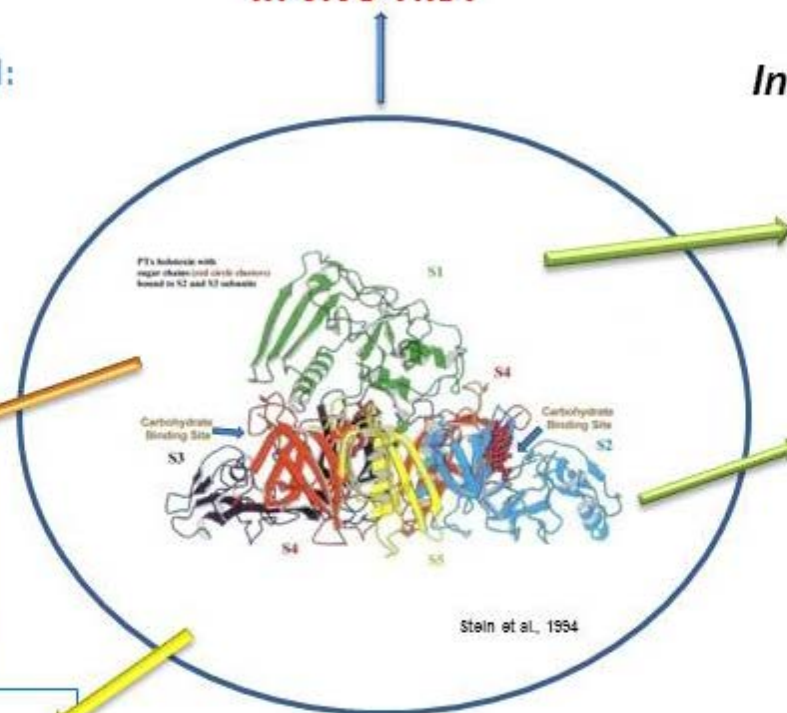
Understanding mechanisms & interactions PTx ± other Ags

Leukocyte-endothelial interactions at BBB:

- Permeability assay
- Adhesion molecules expression
- Cytokine & chemokine release

Toxin translocation:

- Fluorophore-labelled toxin or anti-PT Ab
- Cell – toxin interaction and location
- Confocal Microscopy



*In vitro* biochemical tests

A subunit (S1):  
ADP-ribosylation activity  
Enzyme-HPLC

B subunit (S2-S5):  
Binding activity  
Carbohydrate binding assay

Relationship  
*in vitro/in vivo*

# Materials and Methods

## Vaccines

<b>Manufacturer</b>	<b>Name</b>	<b>EDQM number</b>	<b>Vaccine Components</b>	<b>Adjuvants</b>
<b>GSK</b>	<b>A</b>	48598	n/a	<b>Al(OH)<sub>3</sub></b>
<b>GSK</b>	<b>B</b>	48600	n/a	<b>Al(OH)<sub>3</sub> &amp; AlPO<sub>4</sub></b>
<b>GSK</b>	<b>C</b>	48602	n/a	<b>Al(OH)<sub>3</sub></b>
<b>Sanofi Pasteur Canada</b>	<b>Pediacel</b>	n/a	<b>DTaP-Hib-IPV/a</b>	<b>AlPO<sub>4</sub></b>
<b>Sanofi Pasteur France</b>	<b>Tetraxim</b>	48568	<b>DTaP-IPV</b>	<b>Al(OH)<sub>3</sub></b>
<b>SSI</b>	<b>Toxoid (vial)</b>	47008	<b>aP</b>	<b>Al(OH)<sub>3</sub></b>
<b>SSI</b>	<b>Vaccine (syringe)</b>	47007	<b>DTaP-IPV</b>	<b>Al(OH)<sub>3</sub></b>

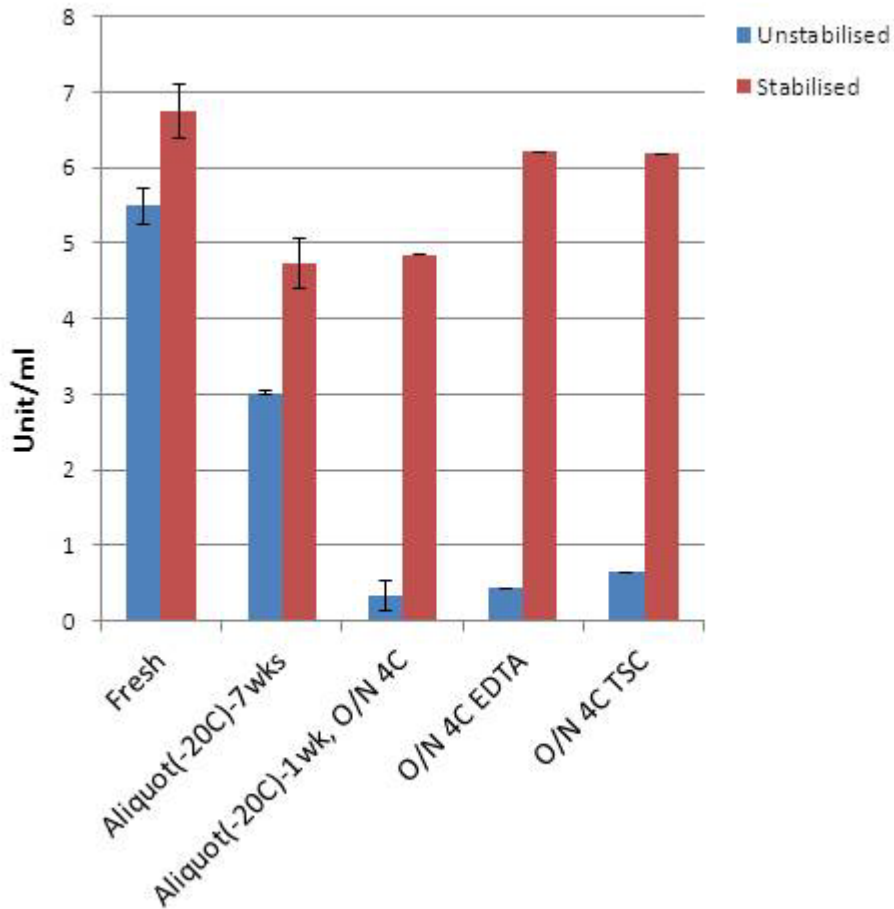
**Spiking Protocol** (Provided) to spike with BRP (EDQM) to final concentrations of 2, 10, and 20 IU/mL

**Desorption of vaccine samples** (optional): with 150mM EDTA or 3.4mM Na Citrate in PBS (Overnight/+4°C)

## Study design

- Requested to perform a minimum of 3 independent assays on 3 different days.
- Each of the spiking concentrations must be assayed in triplicate per assay (a total of 9 data points)
- Each vaccine sample should also be tested without PTx spike in order to have a baseline value.
- All values must be expressed in IU/mL and, where suitable, in % recovery
- Report results in the provided reporting sheet.

# Stability of BRP- Carb-Binding assay

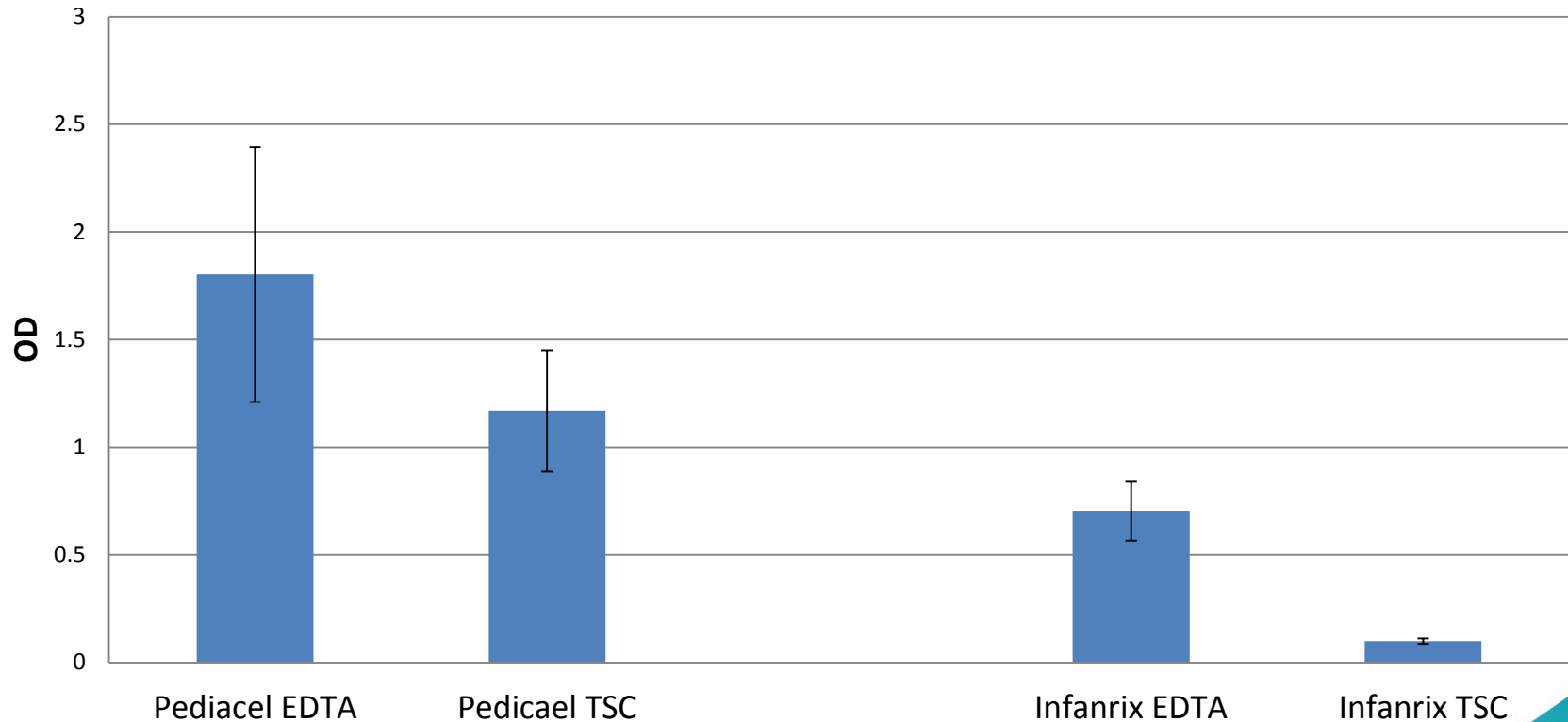


BRP at 10 IU/ml

## Discussion

1. BRP in 50% Glycerol may not be stable under the present storage condition
2. Not due to the desorption buffer(s)
3. 2% (2mg/ml) OVA could be used as stabiliser
4. For long term storage: Freeze-dry in small aliquots?

# Comparison of desorption buffers

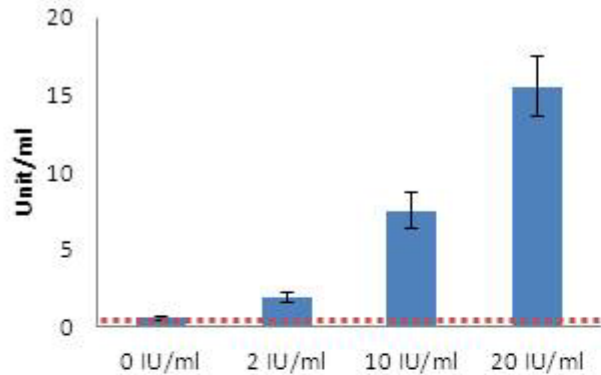


n=3

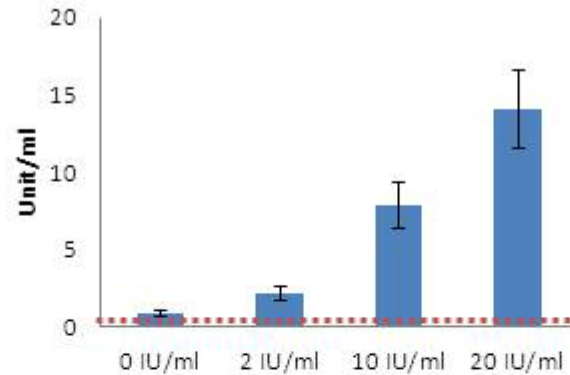
Based on the preliminary results, all the future studies were carried out using EDTA buffer for the desorption.

# Carb-Binding Assay

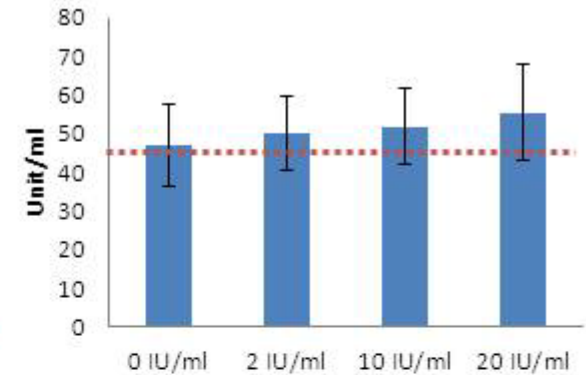
GSK A (48598) n=3x3



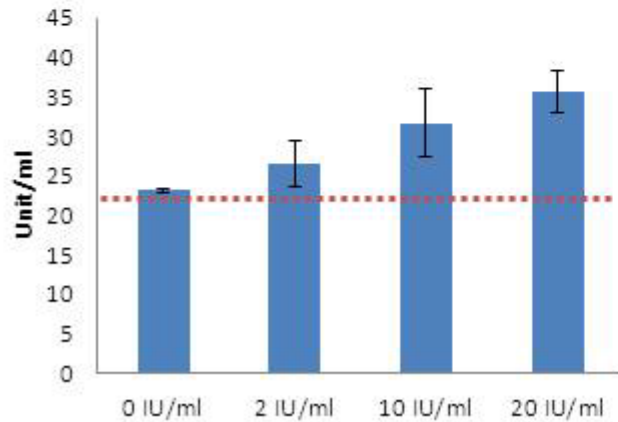
GSK C (48602) n=3x3



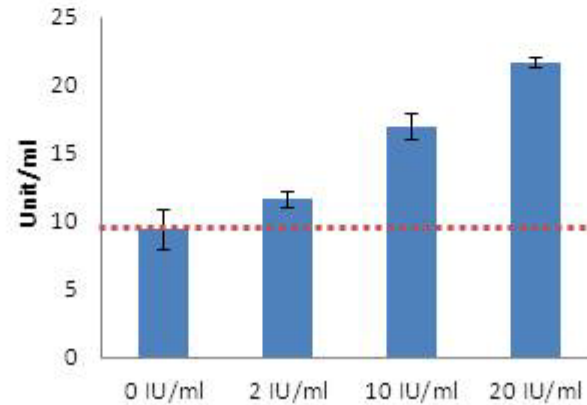
Pediacel (C2855AA) n=3x3



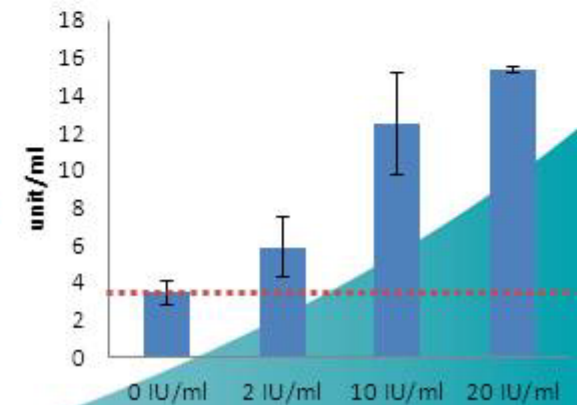
Tetraxim n=1x3



SSI aP n=1x3

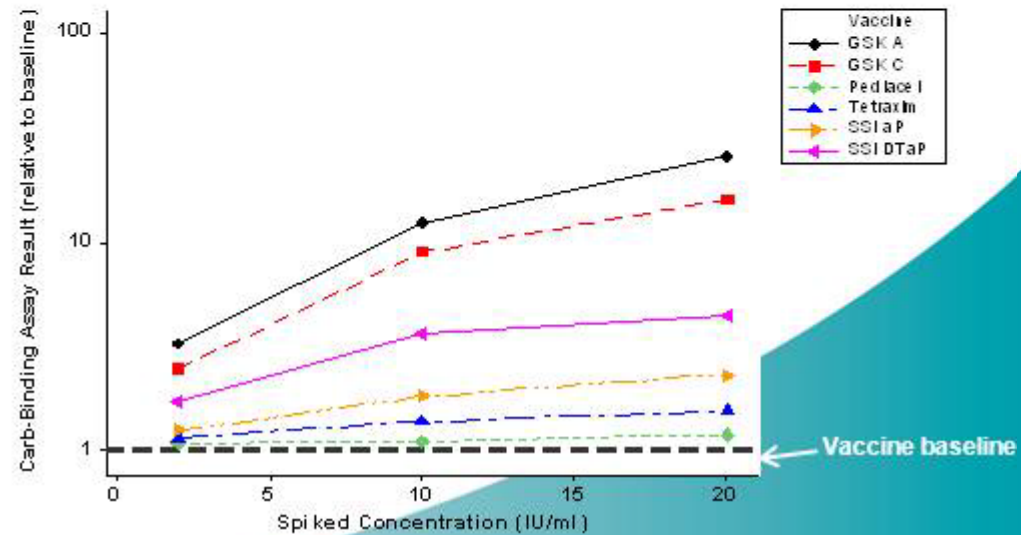
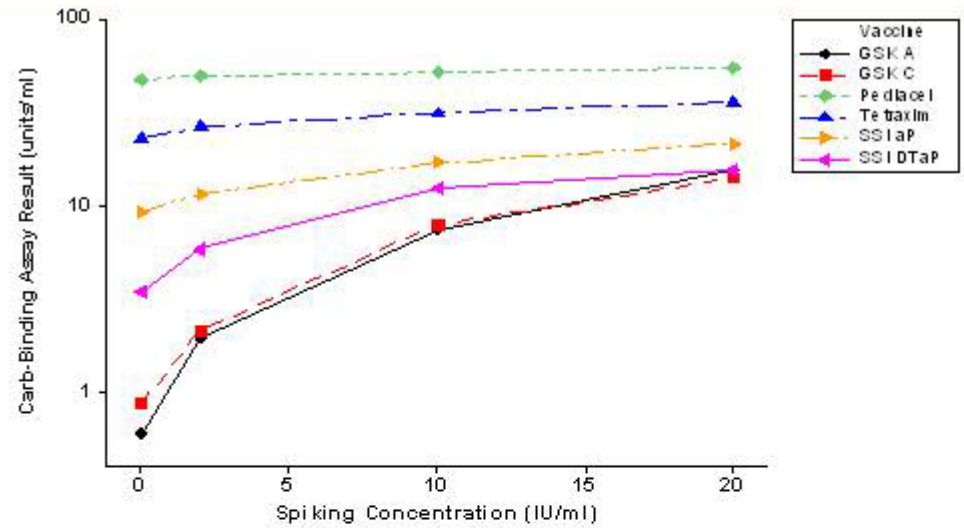
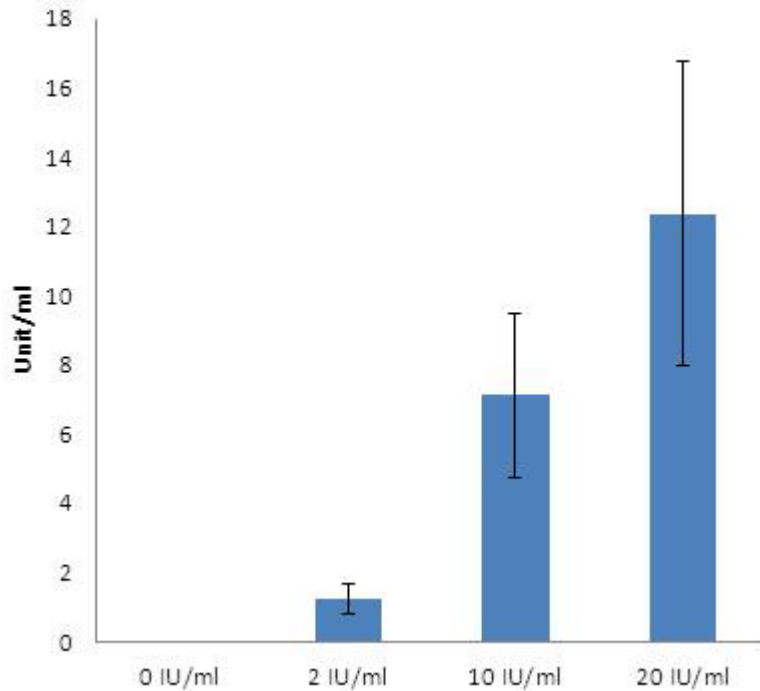


SSI DTaP (47007) n=2x3



# Carb-Binding Assay

BRP n=6x3

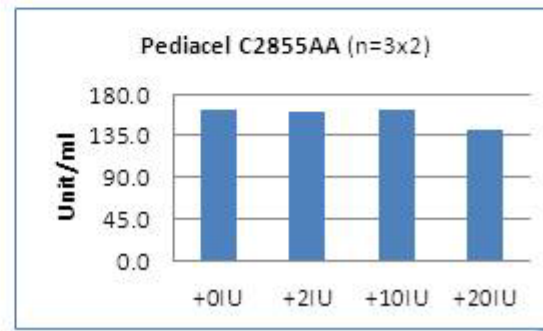
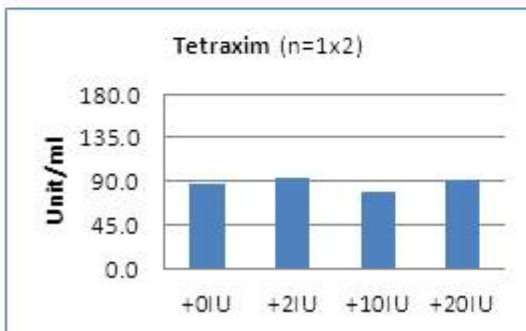
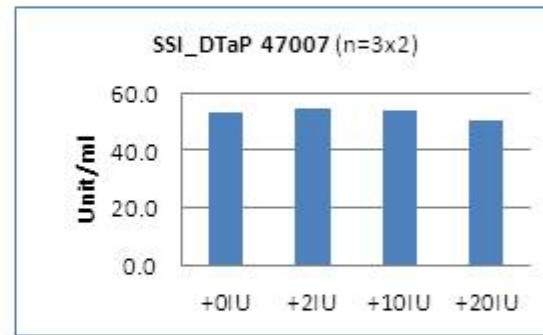
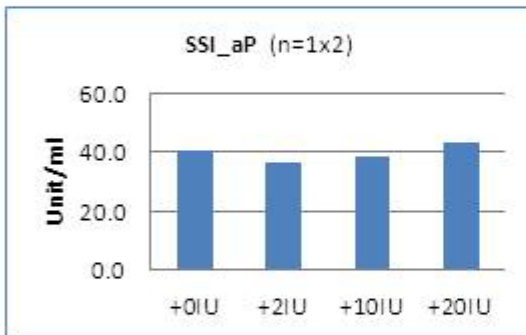
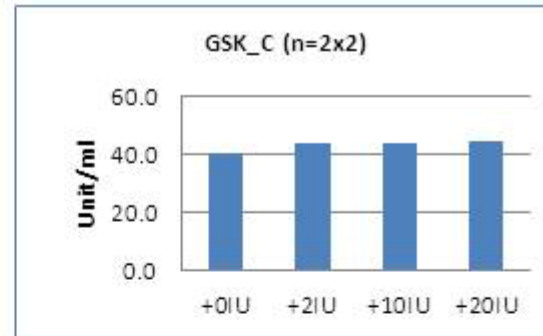
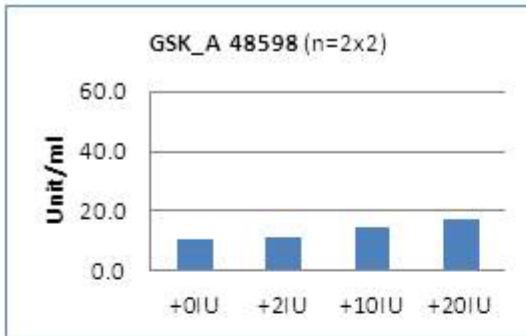


# Discussion: Carb-Binding Assay

- Different types of vaccines showed up to 78 fold difference in their binding activities, ranging from 0.60 - 47.05 unit/ml.
- PT spiked at 2IU/ml was picked up in most cases.
- The differences between the PT doses spiked were not statistically significant for some of the products, e.g. Pediacel.
- The spike dose response magnitudes are product specific & showed very different slopes.
- PTx preparation may not be a good reference for some of the products.



# E-HPLC

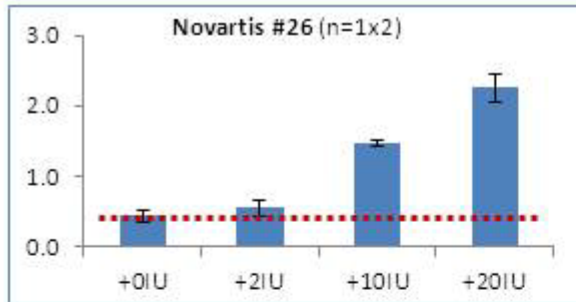


- No dose response for any products except GSK A
- What is/are the problems?
- Any solutions?

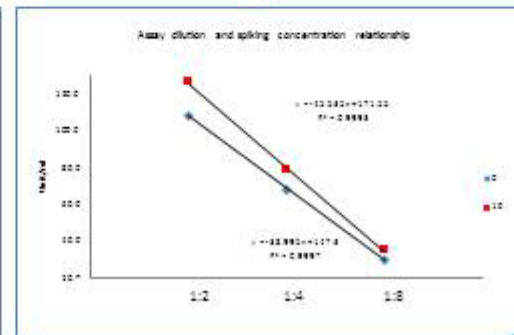
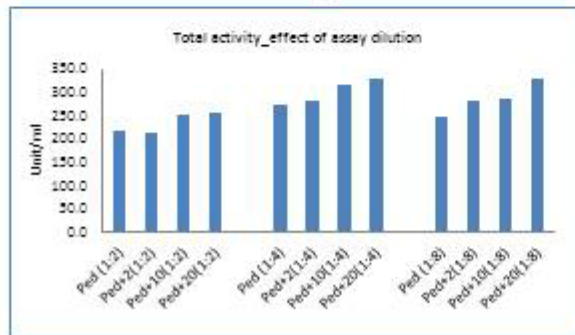
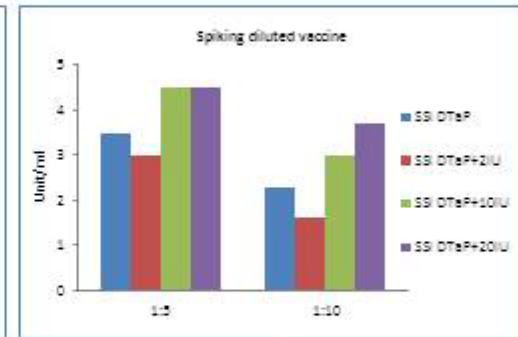
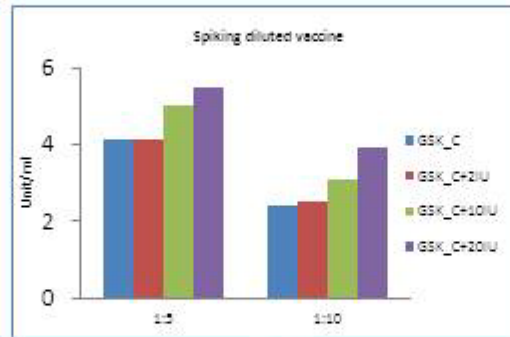
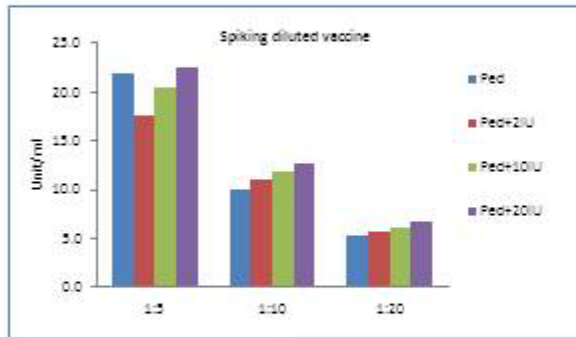
# Factors could influence of the assay

- **Assay sensitivity or accuracy?** Unlikely problem, as GSK A worked.
- **Effect of matrix?**
  - *Interaction*
  - *Concentration*
- **Residual activities presented in the vaccine formulations?**
  - *Vaccines with low activities*
  - *Vaccines with high activities: Could result in masking the small percentage increase in activity by the spiked PTx*
- **Other factors?**
  - *Adsorption/desorption process of PTx –vaccine specific*
  - *?????*

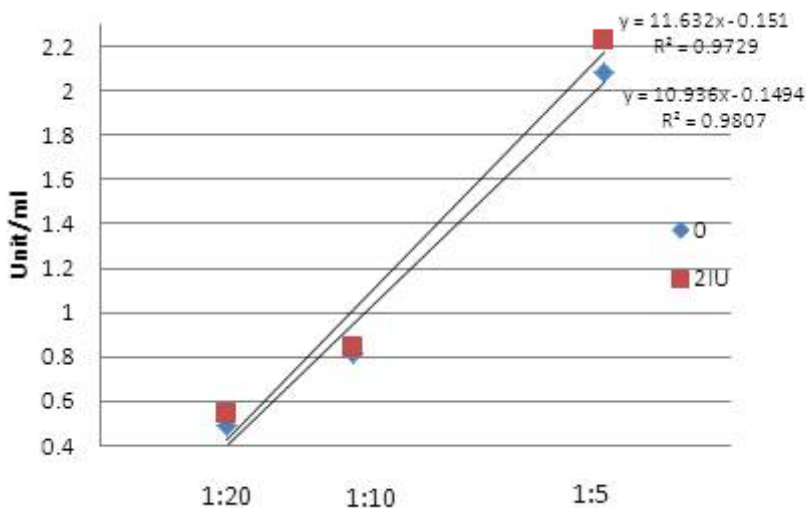
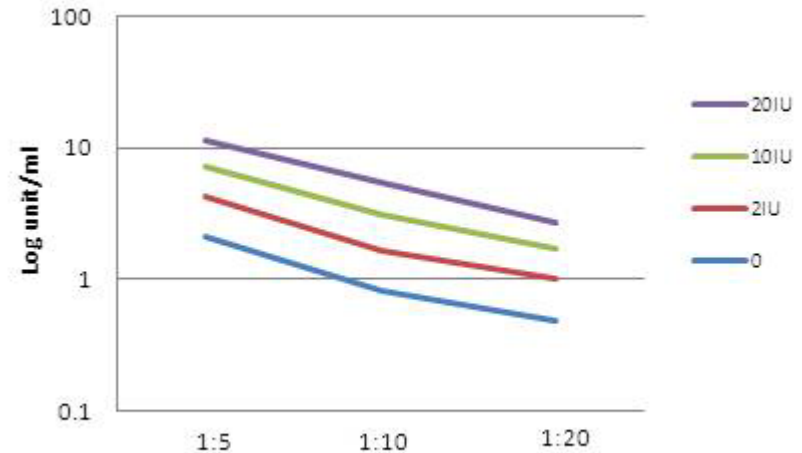
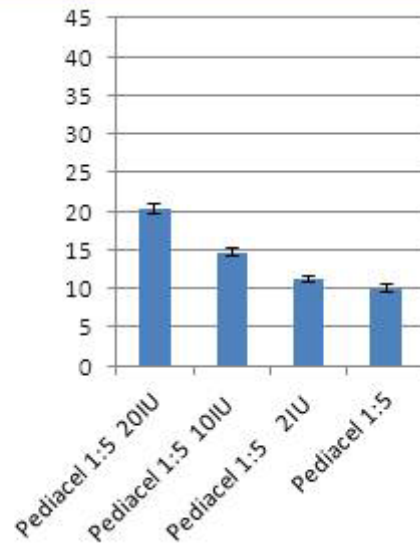
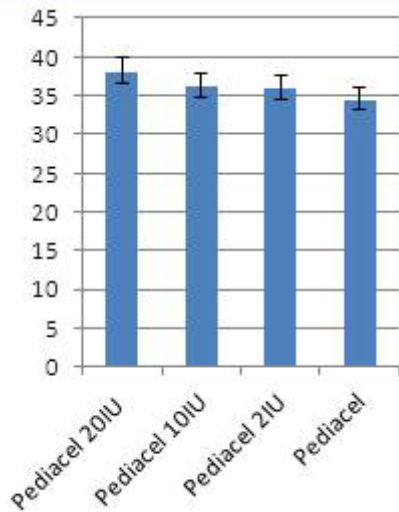
# E-HPLC – Spiking diluted vaccine



1. To test assay sensitivity
2. Spiking of diluted vaccines (to reduce residual baseline activity)
3. Effect of dilution on spiked vaccines (assay environment effect)



# Improved assay of Pediacel as an example by dilution (Carb-binding assay)



## Discussion

- Dilution of vaccine spiked with PTx and then desorption could potentially improve the assay performance
- The dilution factor may be product specific?
- The role of product specific reference vaccine?
- Possibility of using statistical analysis to quantify the activities of test vaccines comparing to the reference vaccine ?

# Discussion

## **This study:**

- PTx BRP was not stable under the 50% glycerol storage/dilution protocol
- Carb-binding assay could detect 2IU spike in most vaccines
- E-HPLC may work only with low activity vaccines
- Assay sensitivities for both *in vitro* assay methods are not a problem
- Our results suggest that the use of recovery of spikes may not be meaningful because of the uncertainty of BRP stability and the effects of matrices/residual activities

## **Possible solutions :**

- Use stabilised PTx for spiking, eg. in 2% Ovalbumin & freeze-dried?
- Use vaccine specific protocols to assay the enzymatic and binding activities eg. assay diluted vaccines especially for those with high baseline activities
- Need to establish vaccine (type or manufacturer) specific assay validation criteria

# The Way Forward - suggestions



1. Establish product specific Ref vaccines based on HIST historical data ?
  
2. Assay parameters to be considered
  - Establish a suitable dilution factor for a specific product ?
  - Use spiking assay to establish dose response curve for the reference vaccine ?
  - Desorption condition if required ?
  - Assay validity criteria:
    - an allowable range of a positive control vaccine to PTx at chosen dose(s) ?
    - define lowest detection limit ?
    - Specification for a product to pass?
  
  - Others???

# The Challenge



**How to make sure the *in vitro* assay is comparable to HIST??**