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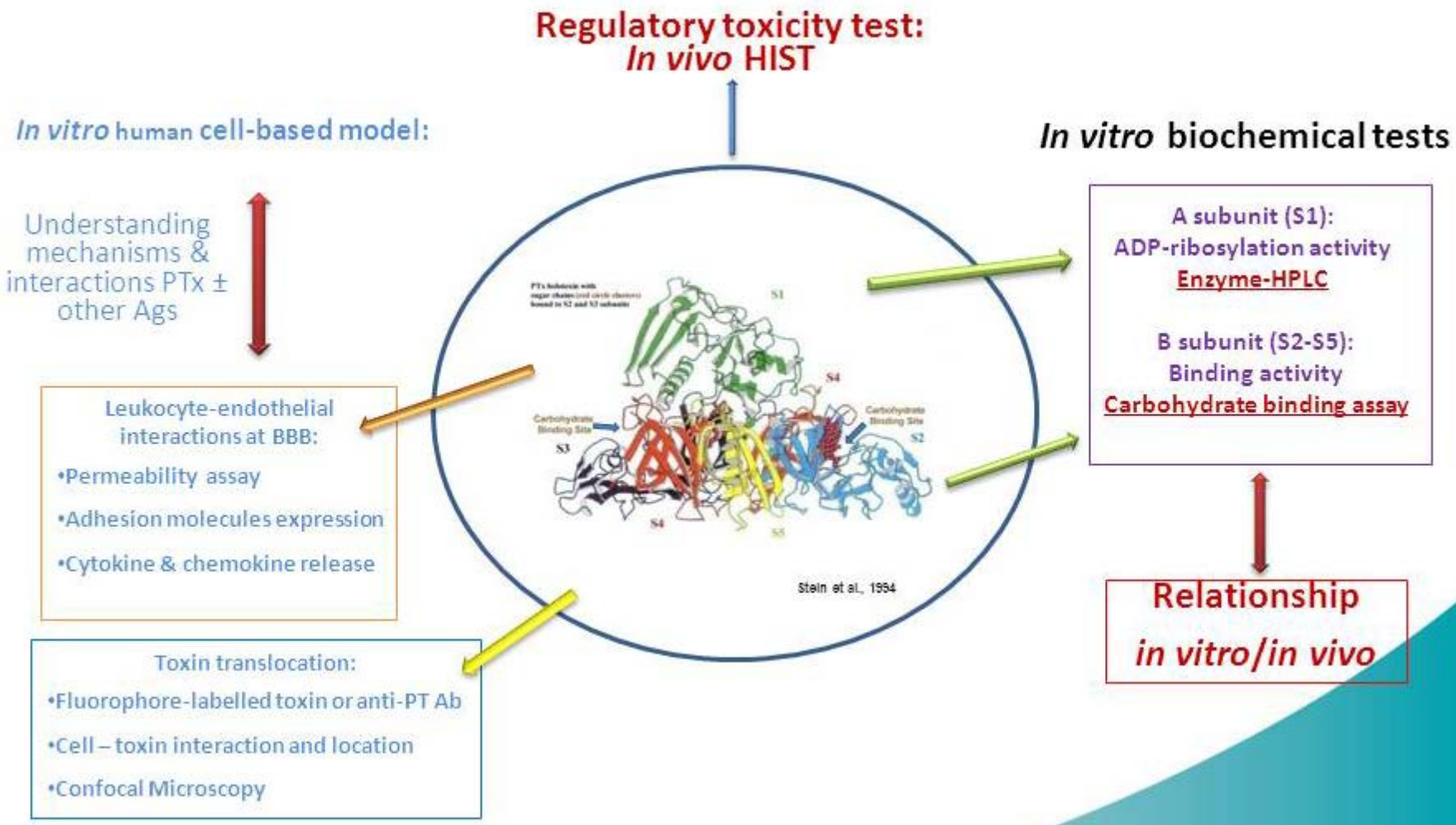
National Institute for Biological Standards and Control
Assuring the quality of biological medicines

Introduction



Materials and Methods

Vaccines

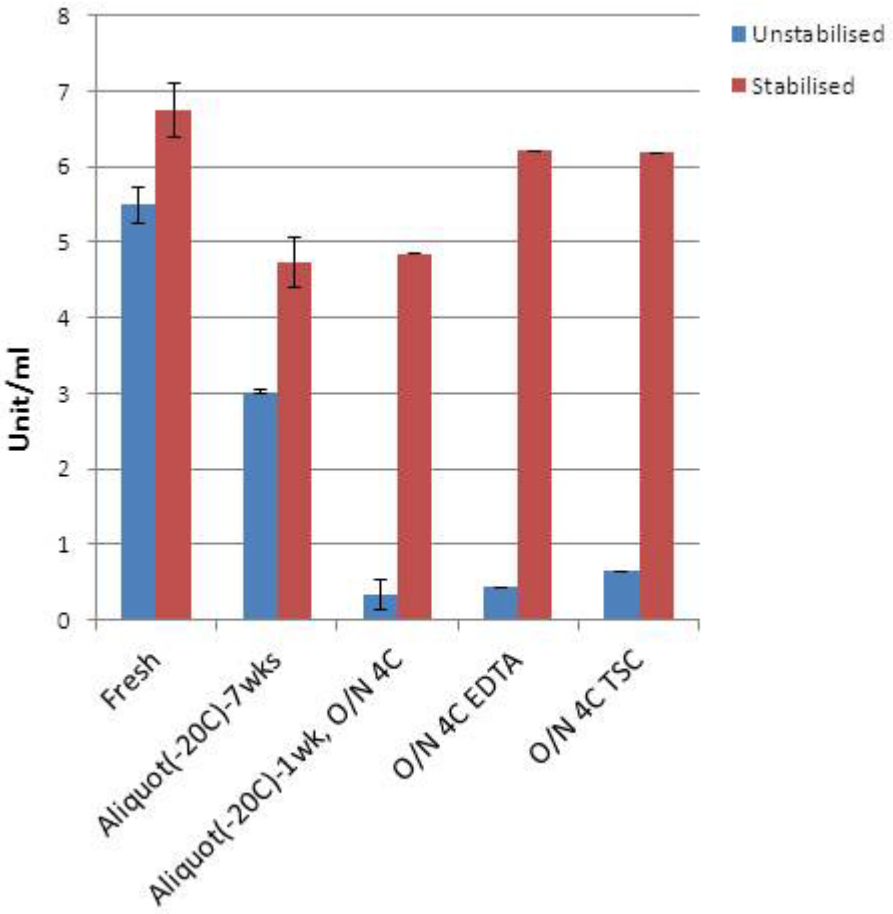
Manufacturer	Name	EDQM number	Vaccine Components	Adjuvants
GSK	A	48598	n/a	Al(OH)3
GSK	B	48600	n/a	Al(OH)3 & AlPO4
GSK	C	48602	n/a	Al(OH)3
Sanofi Pasteur Canada	Pediacel	n/a	DTaP-Hib-IPV/a	AlPO4
Sanofi Pasteur France	Tetraxim	48568	DTaP-IPV	Al(OH)3
SSI	Toxoid (vial)	47008	aP	Al(OH)3
SSI	Vaccine (syringe)	47007	DTaP-IPV	Al(OH)3

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

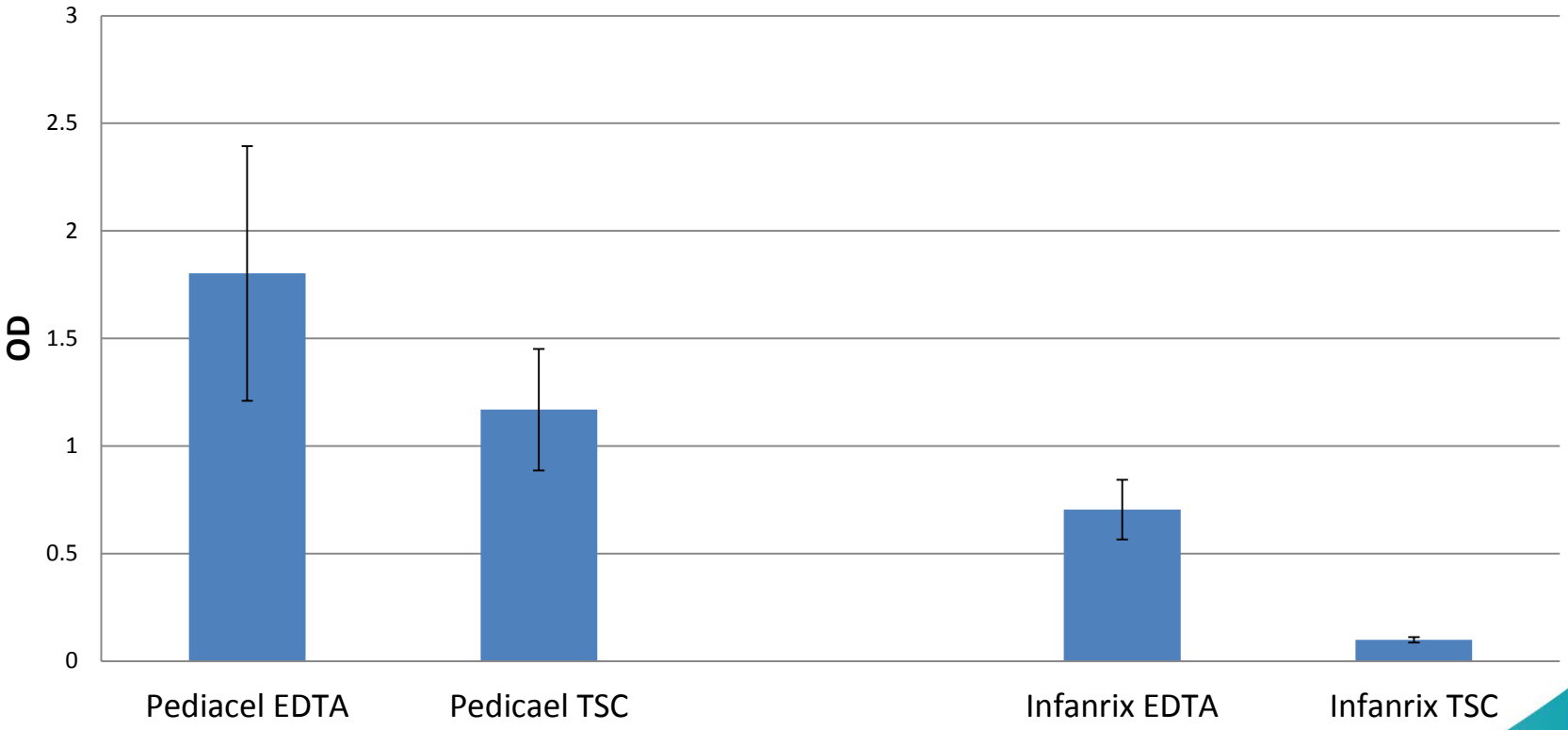


BRP at 10 IU/ml

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?

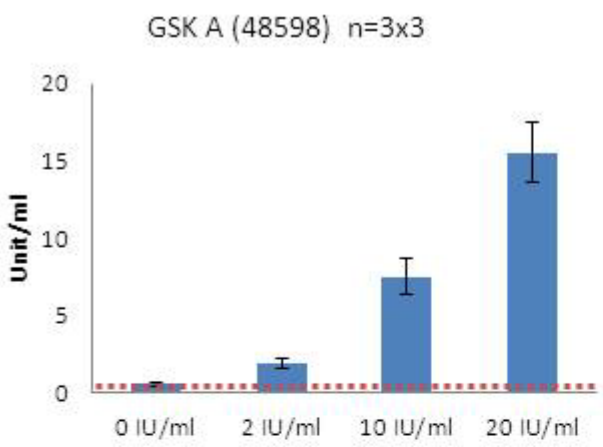
Comparison of desorption buffers

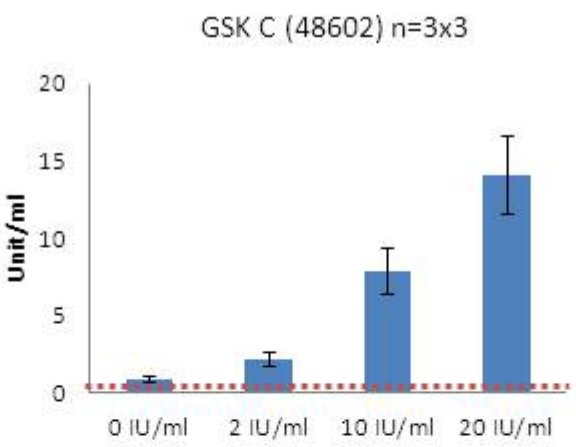


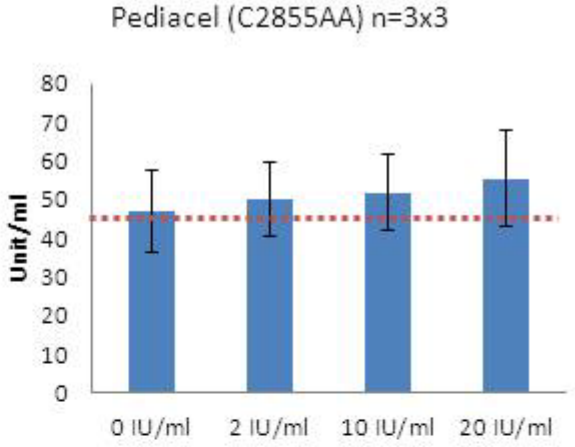
n=3

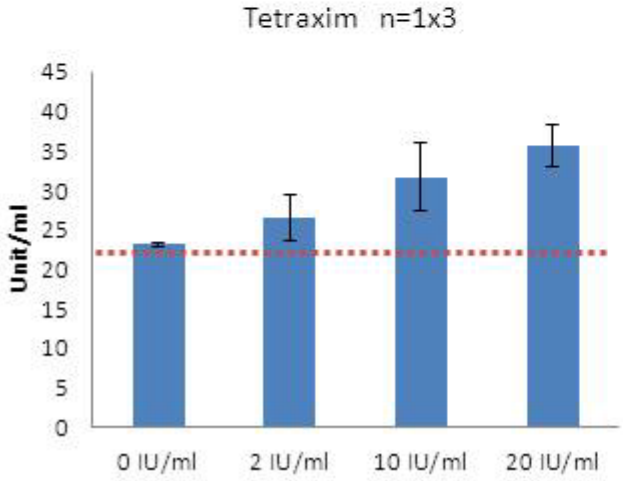
Based on the preliminary results, all the future studies were carried out using EDTA buffer for the desorption.

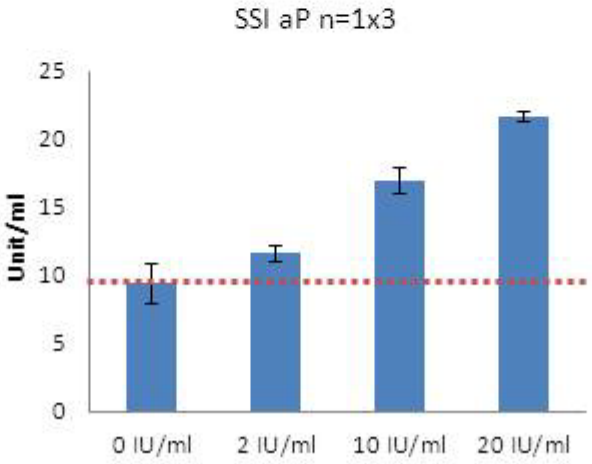
Carb-Binding Assay

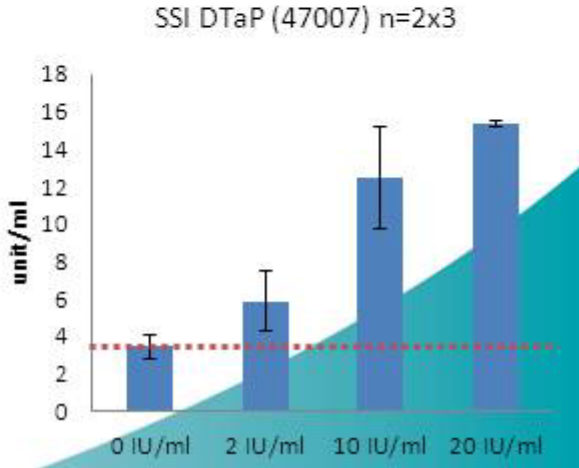




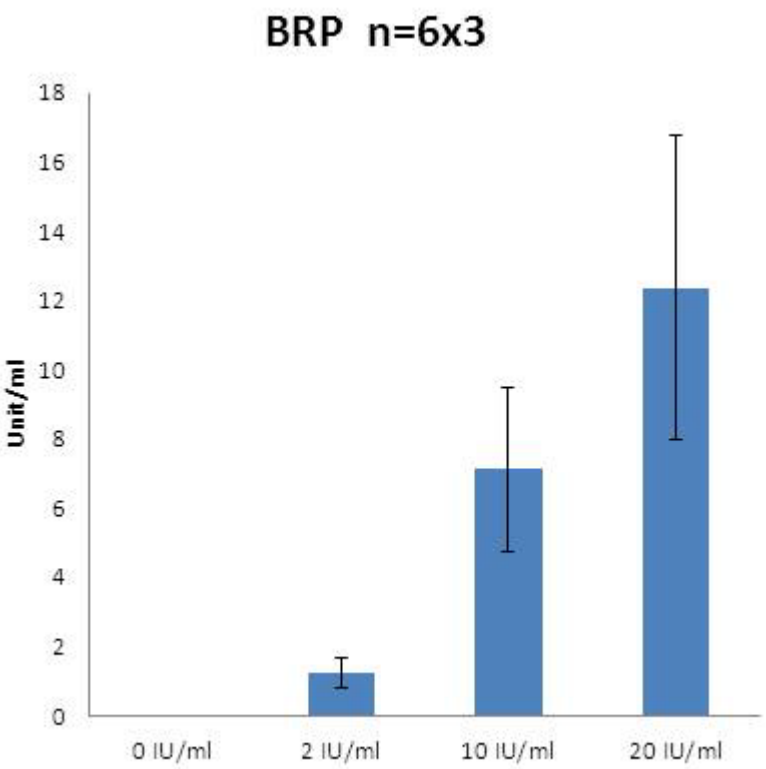


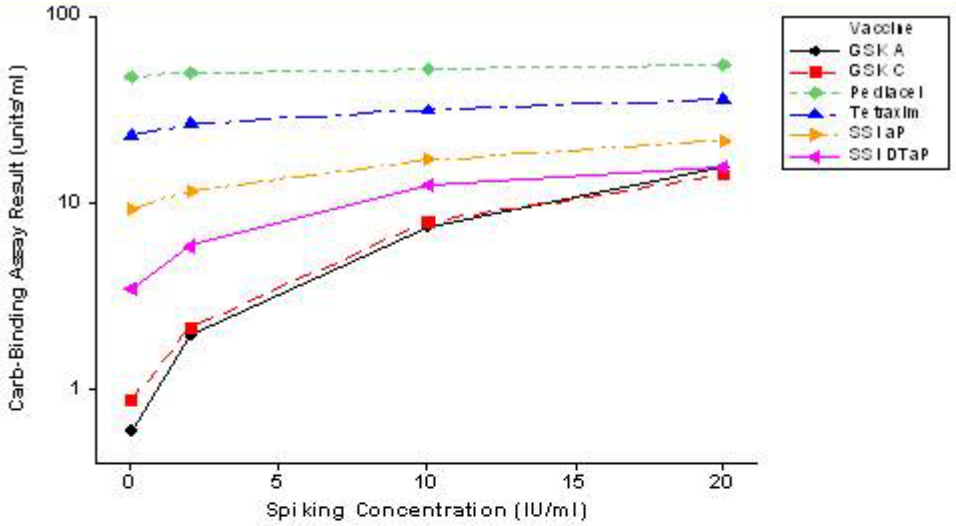


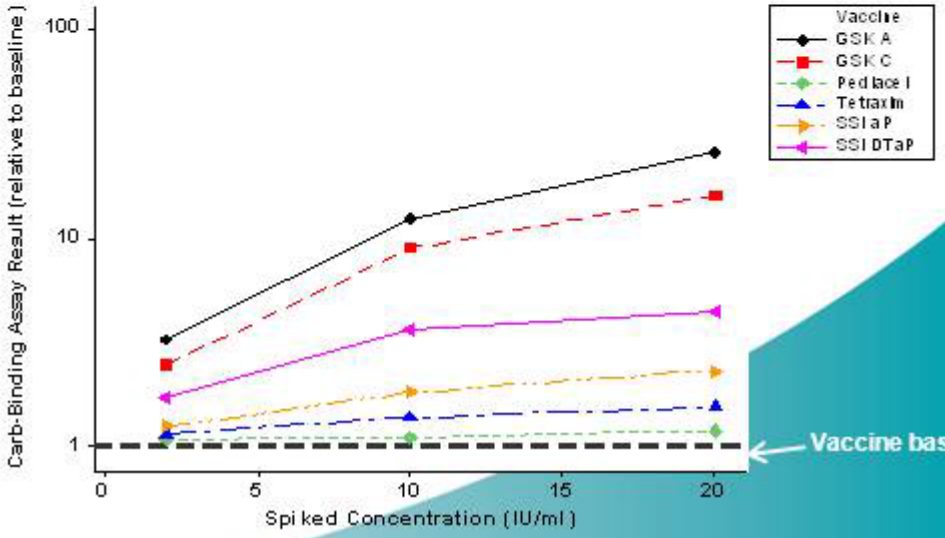




Carb-Binding Assay



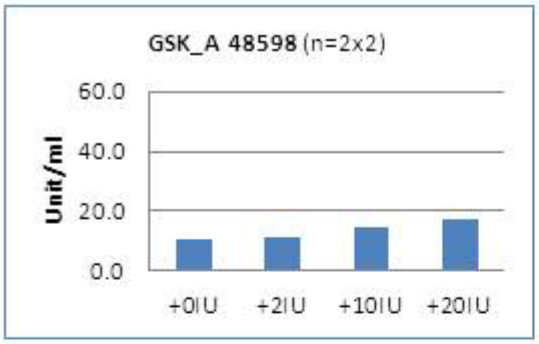


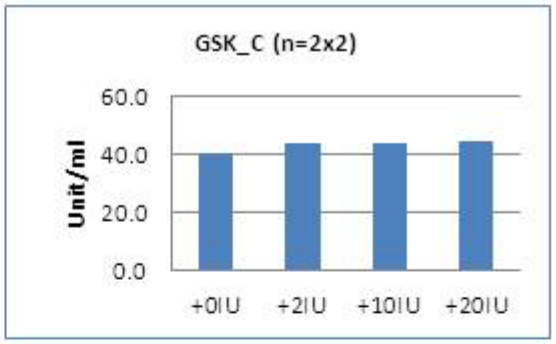


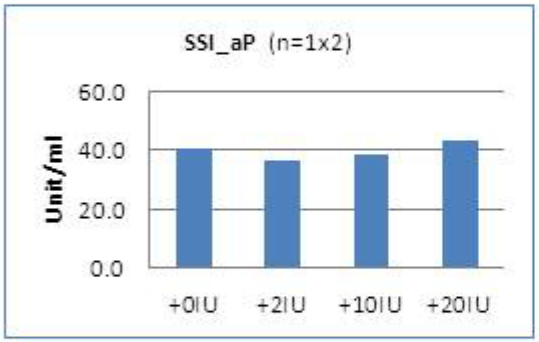
Discussion: Carb-Binding Assay

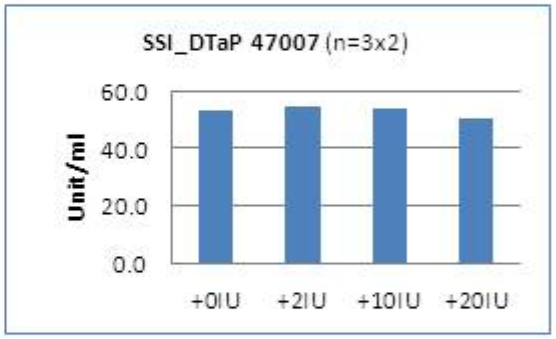
- Different types of vaccines showed up to 78 fold difference in their binding activities, ranging from 0.60 - 47.05 unit/ml.
- PT spiked at 2IU/ml was picked up in most cases.
- The differences between the PT doses spiked were not statistically significant for some of the products, e.g. Pediacel.
- 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.

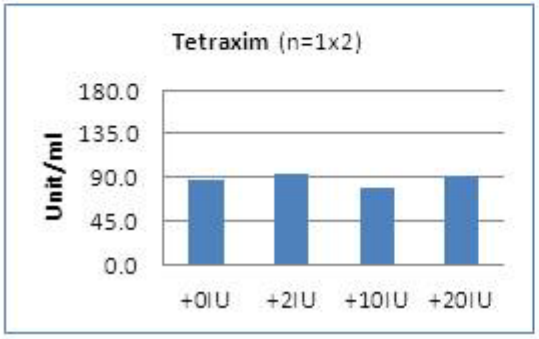
E-HPLC

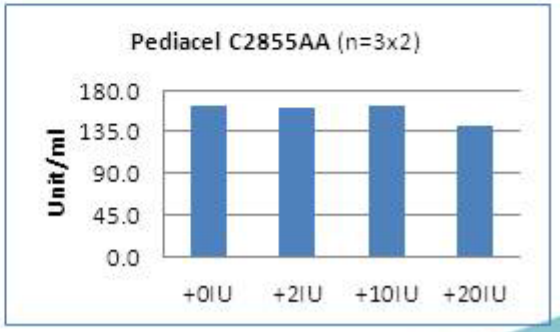










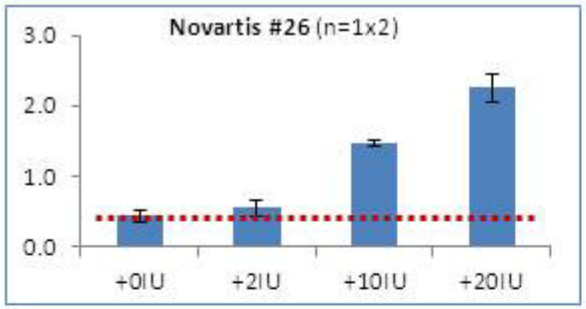


- No dose response for any products except GSK A
- What is/are the problems?
- Any solutions?

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*
  - *?????*

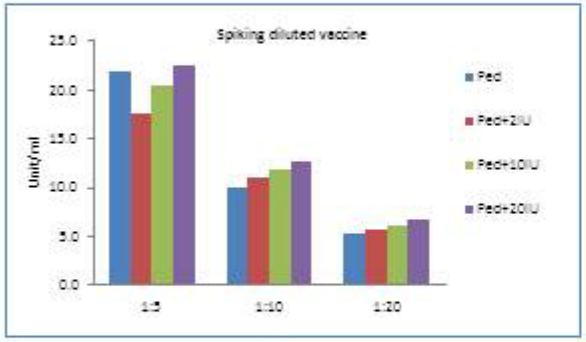
E-HPLC – Spiking diluted vaccine

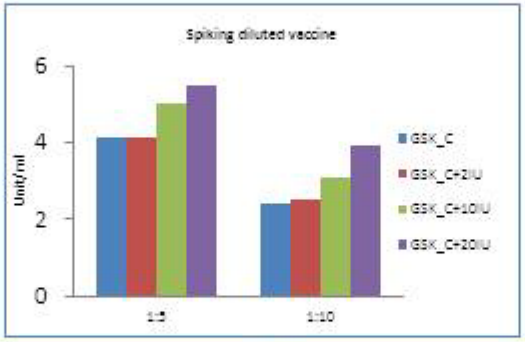


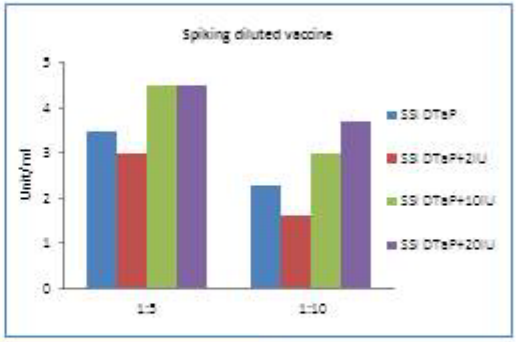
1.To test assay sensitivity

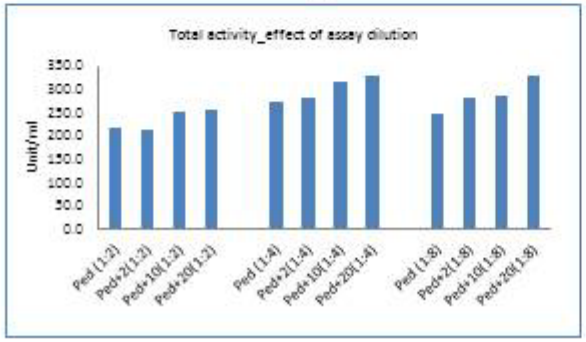
2.Spiking of diluted vaccines (to reduce residual baseline activity)

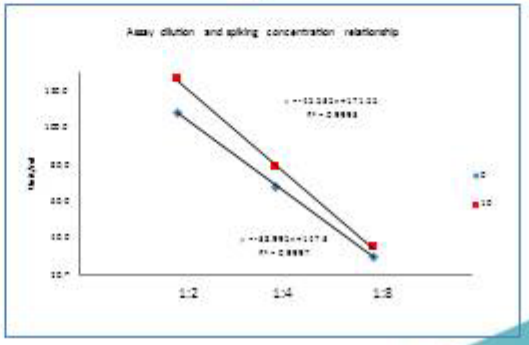
3.Effect of dilution on spiked vaccines (assay environment effect)





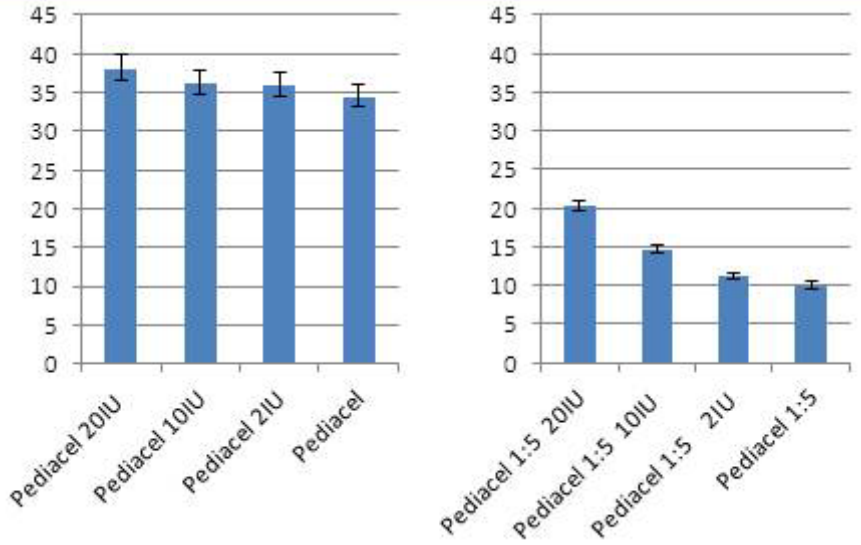


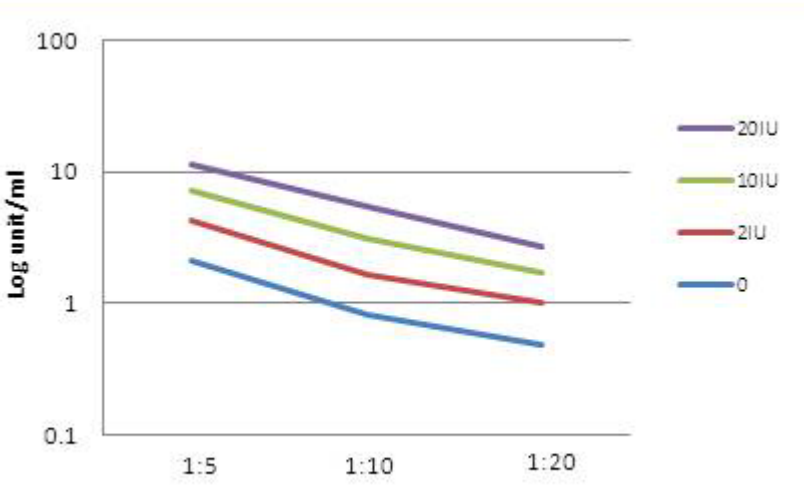


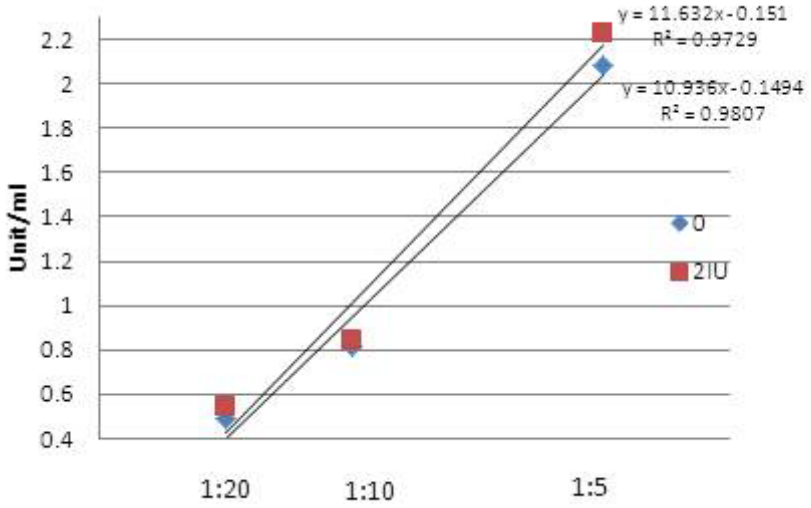


Improved assay of Pediacel as an example by dilution

(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. <u>Assay parameters to be considered</u>
   - 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

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Health Protection Agency

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