PTx detection in spiked aP vaccines using modified CHO clustering and binding assays

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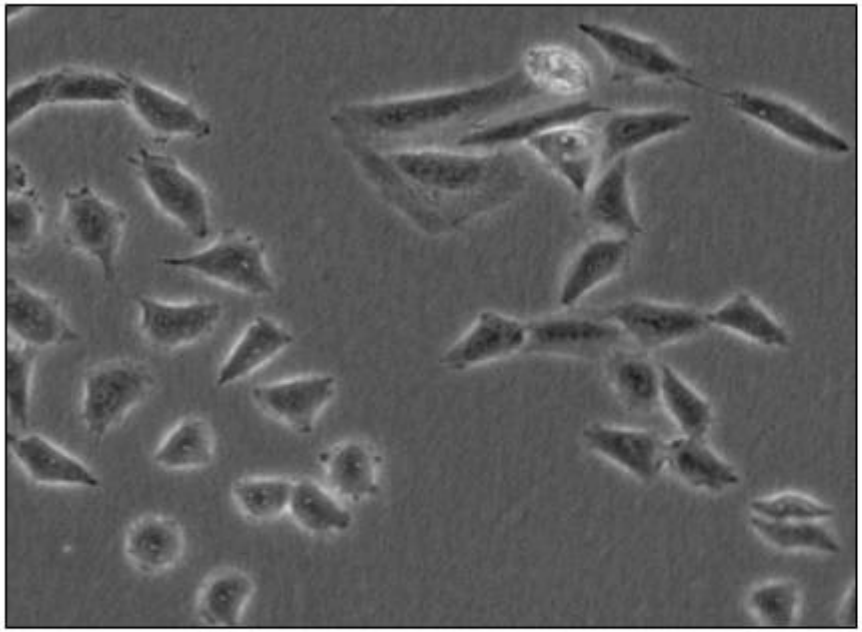
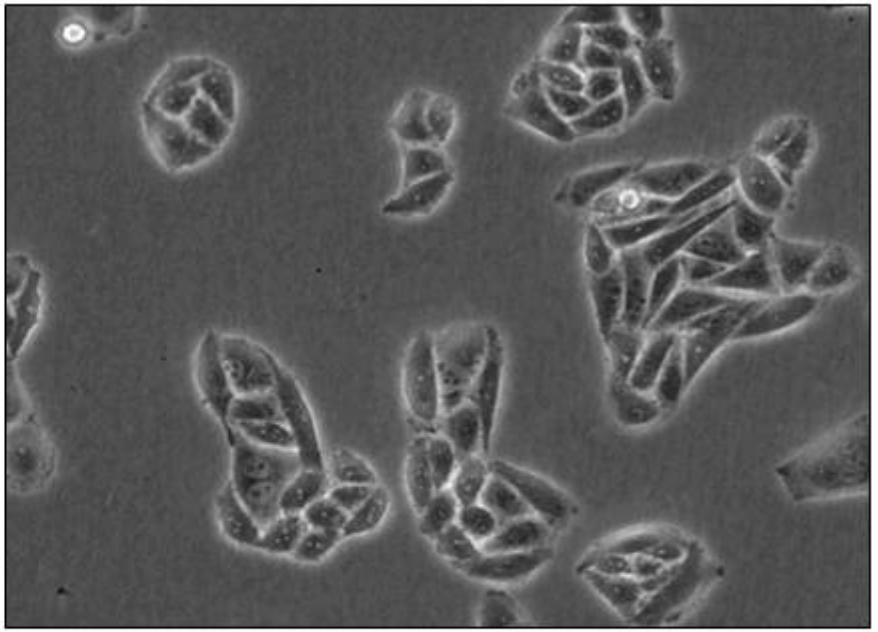
CHO Cell Clustering Assay



Control (no PTx)



+ PTx (48 hrs)

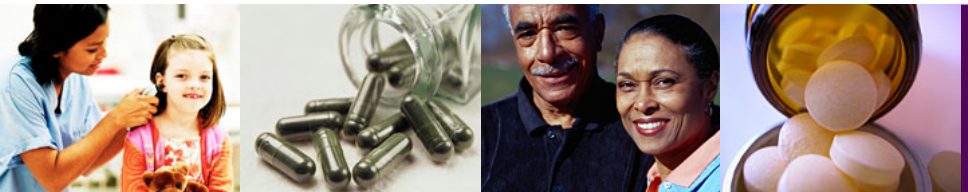
Score: - [+/- , + , ++] +++




CHO Cell Clustering Assay

Ph. Eur. Pertussis Toxin (BRP batch 1)

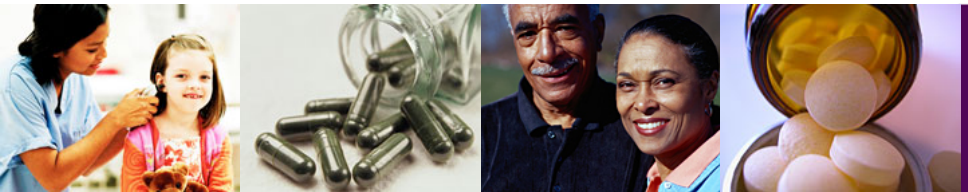
	PTx Conc. (IU / ml)				
Observer	0.02	0.04	0.08	0.15	0.3
1	-	+	++	++	+++
2	-	+/-	++	+++	+++



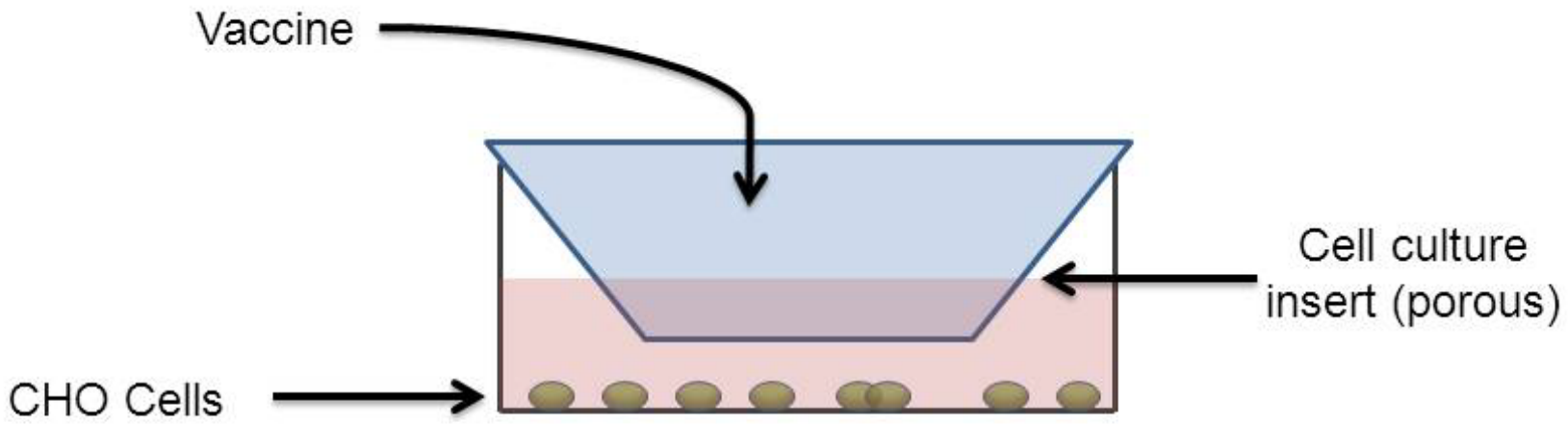
CHO Cell Clustering Assay

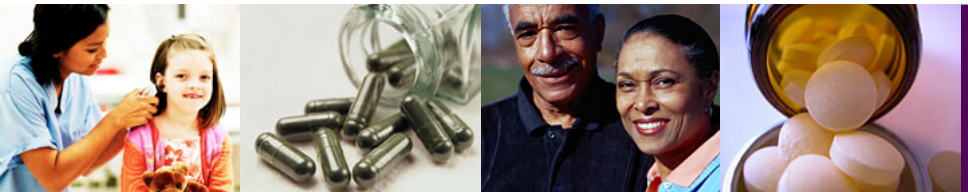
- Used for testing PTd bulk to ensure completion of toxoiding process
- Cannot be used in final product/vaccine due to cytotoxicity of alum adjuvant

- Mechanism of alum adjuvant cytotoxicity?
- Depolarization of cell membranes in contact with adjuvant (?)



Modified CHO Cell Clustering Assay

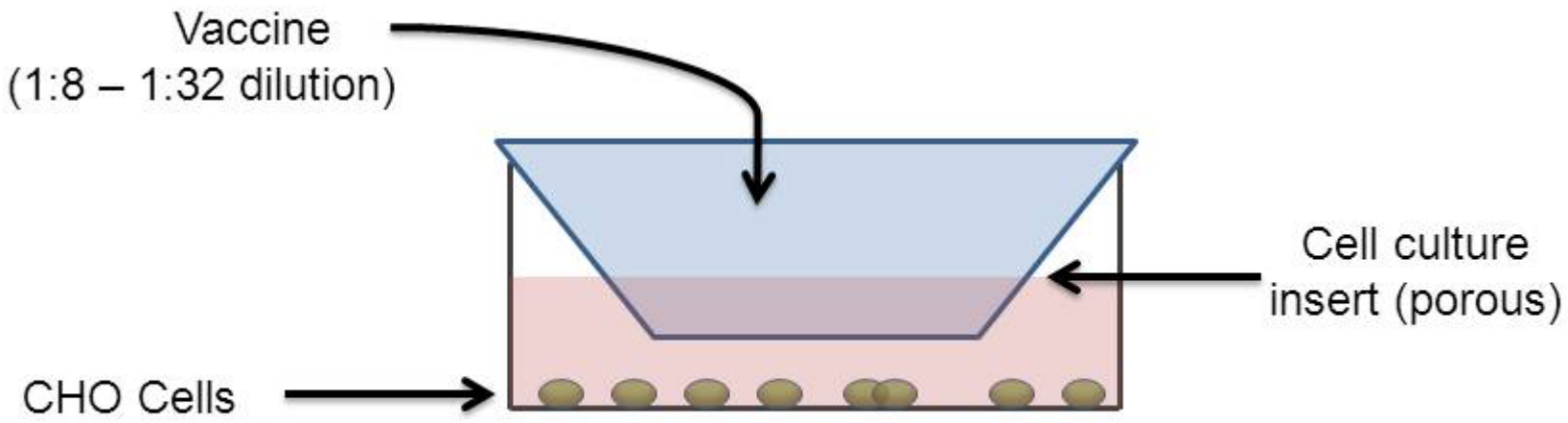




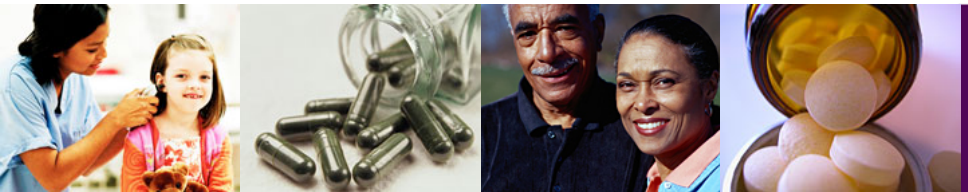
Cell Culture Inserts



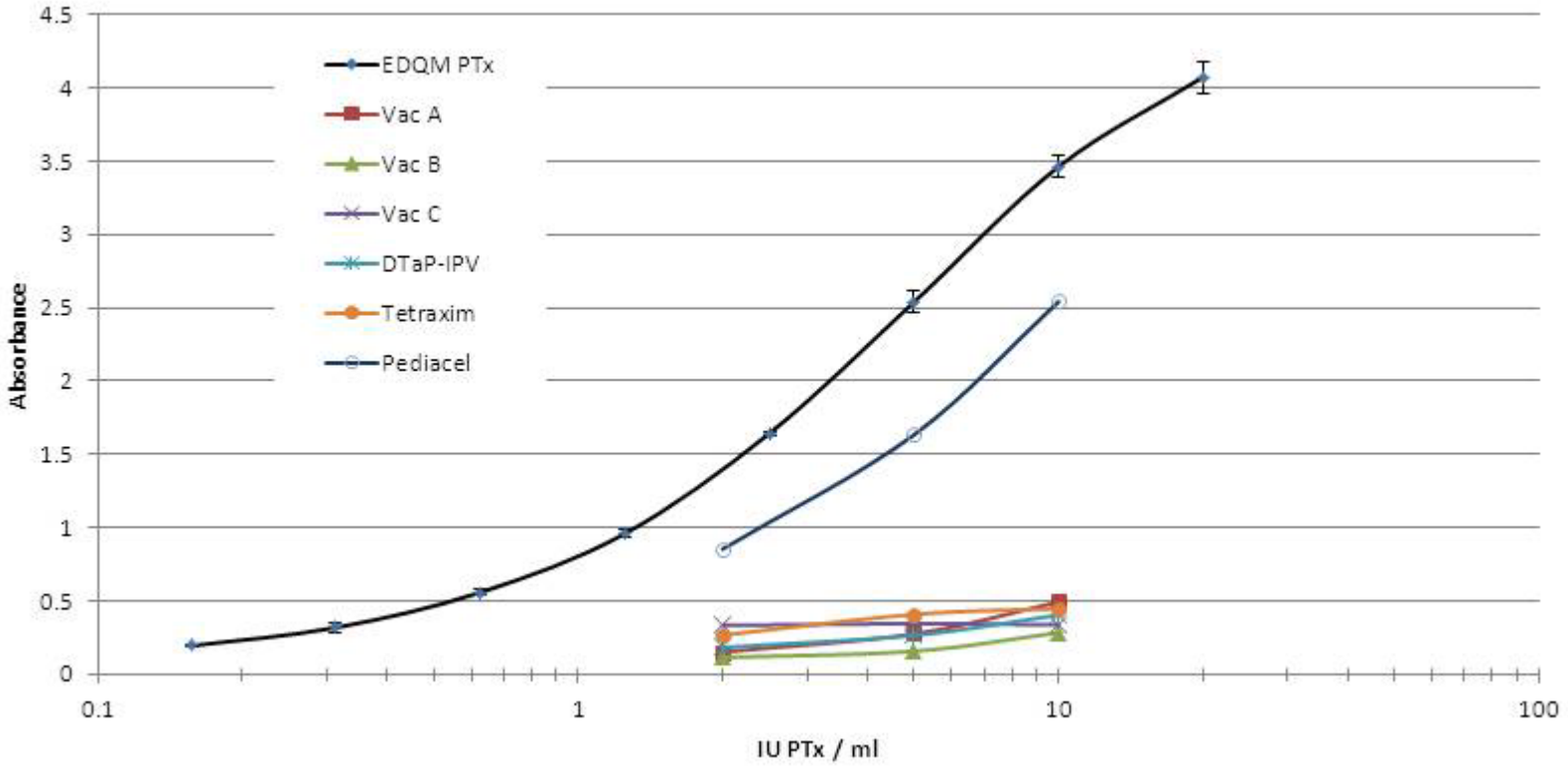

Modified CHO Cell Clustering Assay

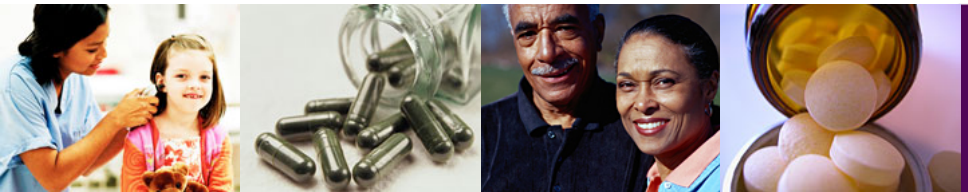


Sensitivity: 5-10 IU PTx / ml vaccine

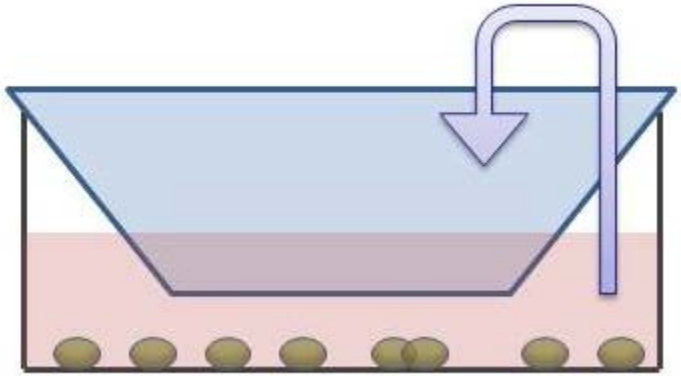


Adsorption of PTx to vaccines (Supernatant of PTx-spiked vaccines)

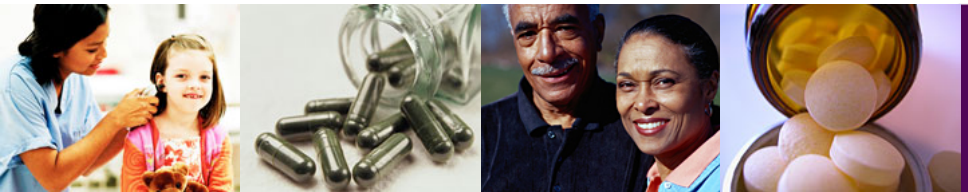




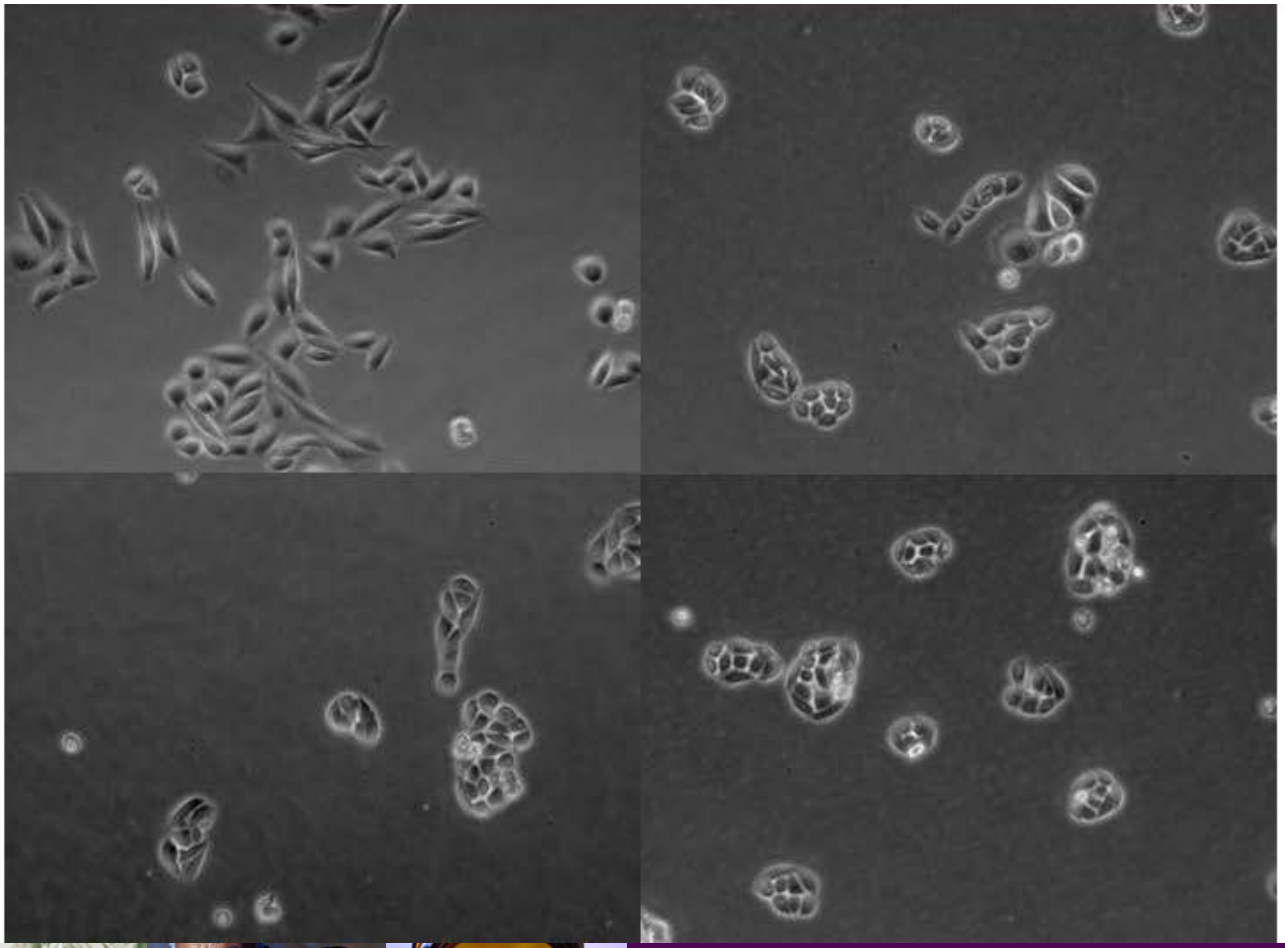
Re-Modified CHO Cell Clustering Assay

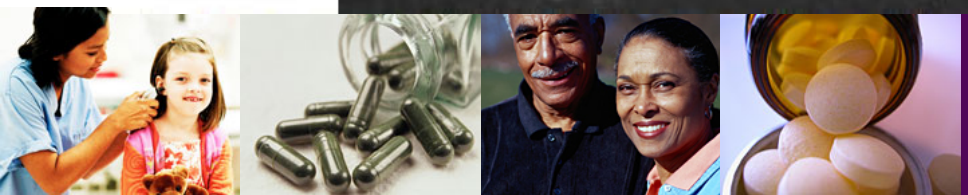


1. Start CHO cell culture and add culture insert.
2. Spike vaccine, adsorb 1 hour
3. Centrifuge vaccine, collect adjuvant
4. Resuspend adjuvant in media, put in culture insert
5. Culture 48 hours, with media circulation at 24 hours
6. Remove insert and score clustering



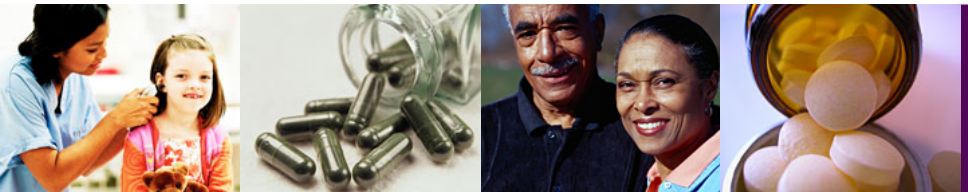
Vaccine A





Modified CHO Cell Clustering Assay

	PTx (IU / ml)			
Vaccine	0	1	2	5
Vac. A	-	++	+++	+++
	+/-	++	+++	+++
Vac. B	-	++	+++	+++
	-	++	+++	+++
Vac. C	+/-	++	+++	+++
	+/-	+	+++	+++



Modified CHO Cell Clustering Assay

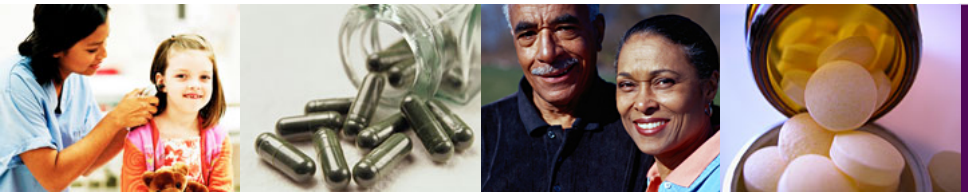
	PTx (IU / ml)			
Vaccine	0	1	2	5
Pediacel	+	+++	+++	+++
	+/-	+++	+++	+++
Tetraxim	-	++	+++	+++
	-	++	+++	+++
DTaP-IPV	-	++	+++	+++
	-	++	+++	+++



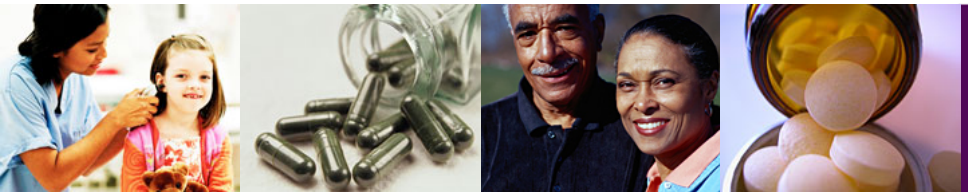

Modified CHO Cell Clustering Assay

Summary:

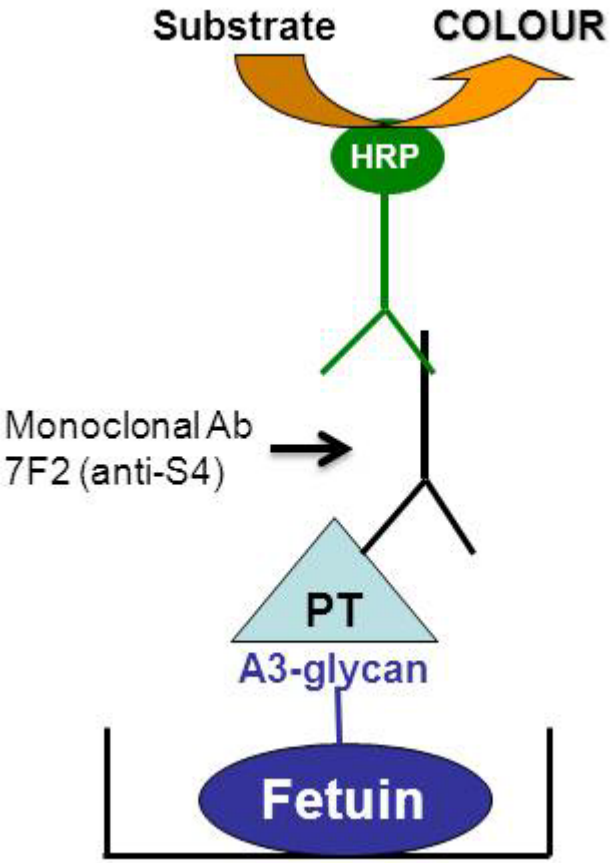
- CHO cell clustering assay can be done on adjuvented vaccines with the use of a culture insert
- Sensitivity is improved by testing adsorbed adjuvant only, and not whole vaccine
- Can detect 1-2 IU PTx/ml vaccine

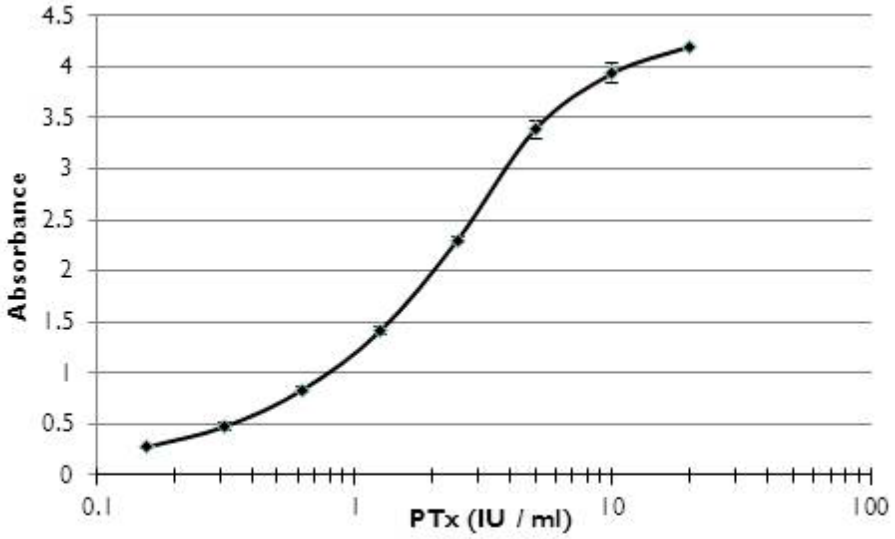


Fetuin Binding Assay:
Recovery of PTx by desorption of spiked vaccines

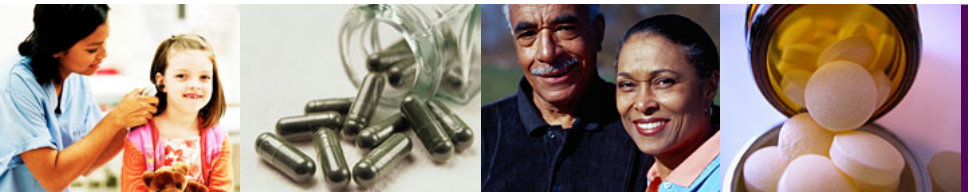


Fetuin Binding Assay

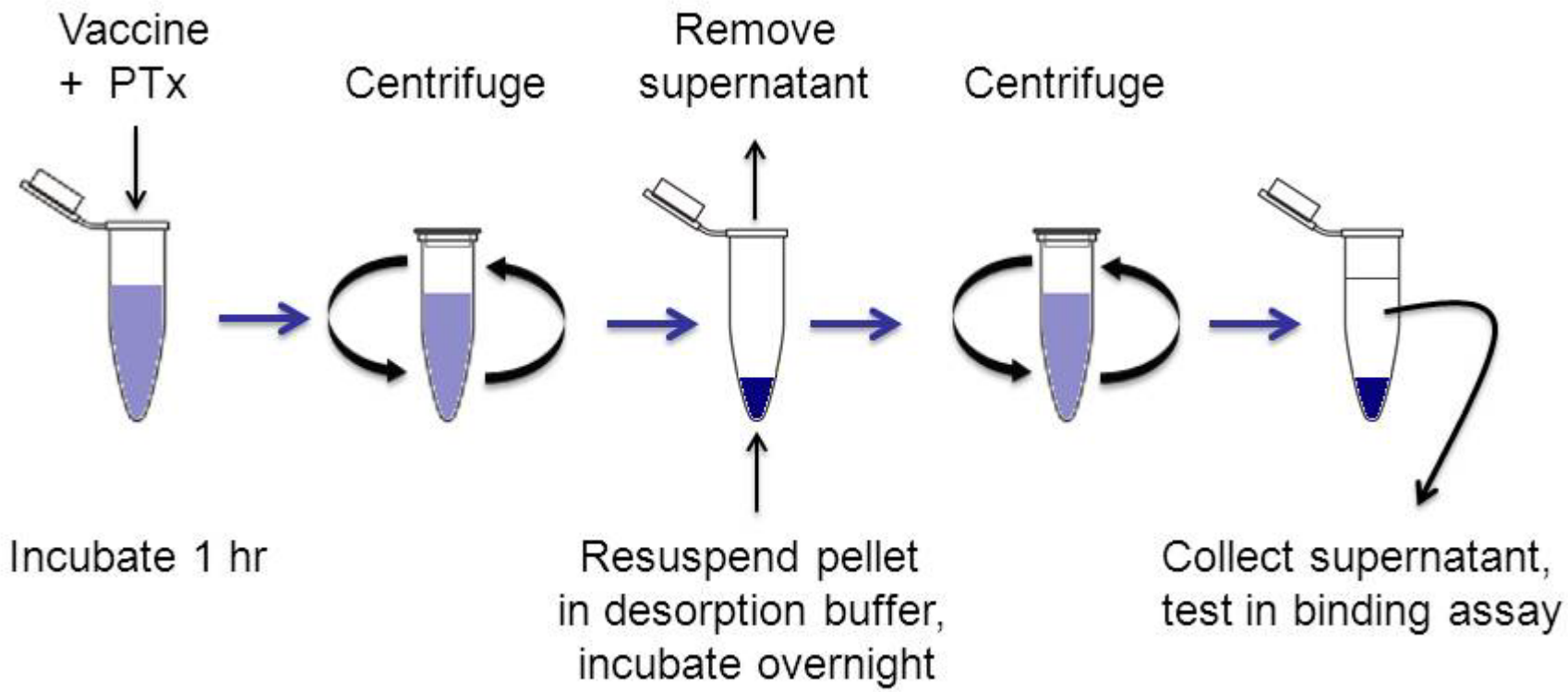


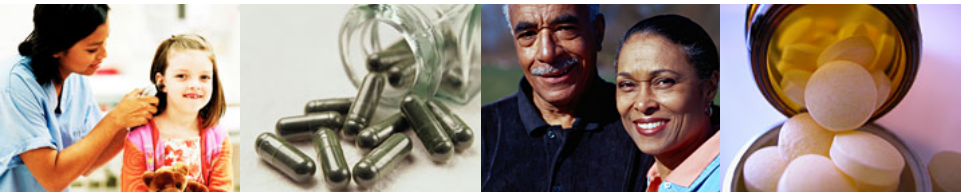


LOQ (in PBS) = 0.2 – 0.4 IU/ml



Fetuin Binding Assay – preparation of samples





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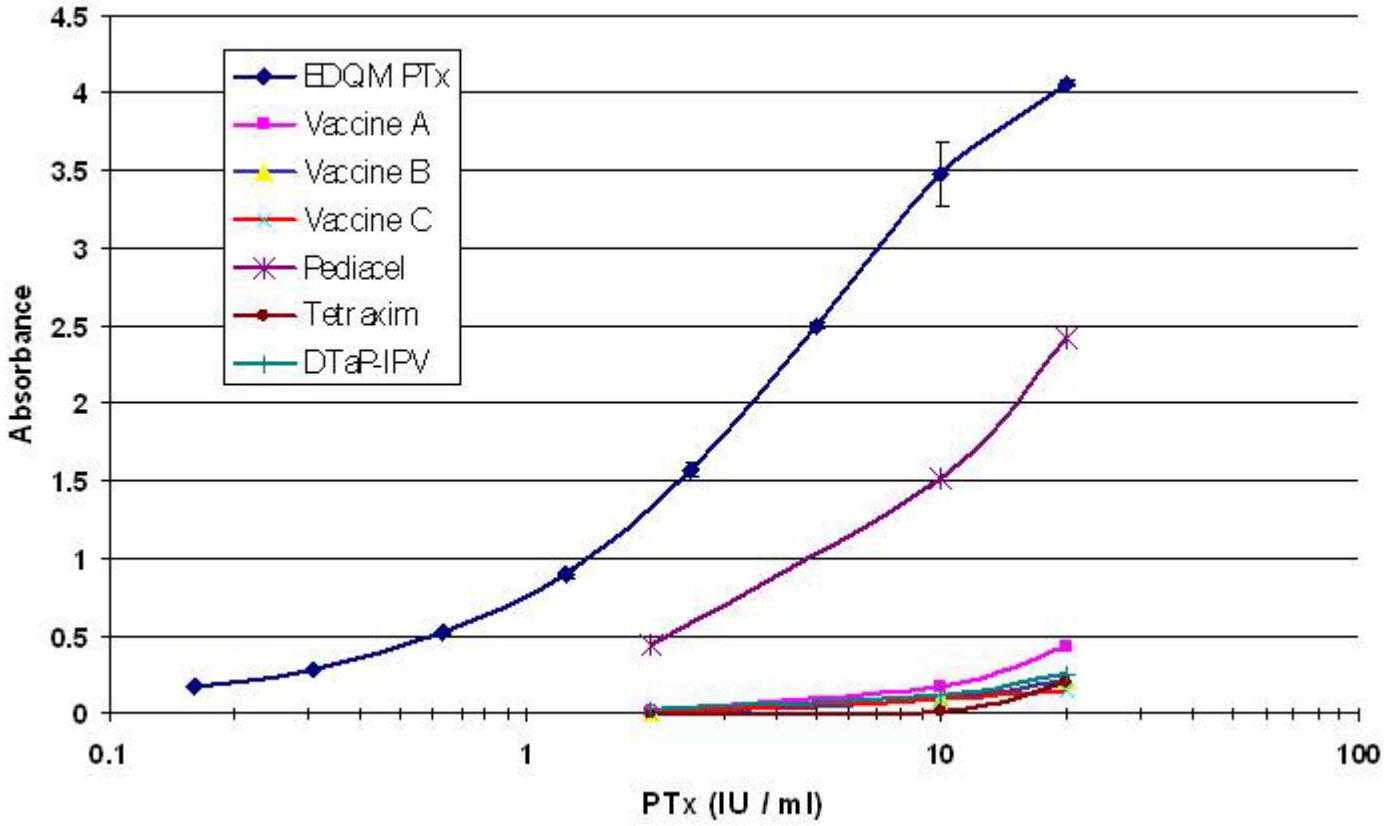
Recovery of PTx by desorption of spiked vaccines



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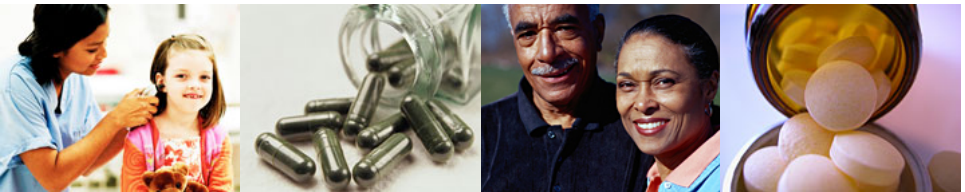
Desorption methods

1. Competition for adjuvant
   - ~~Protein/peptide fragments~~
2. Dissolution of adjuvant
   - Citrate
   - NaOH (and neutralization with citrate)
3. Dissociation from adjuvant
   - EDTA
   - ~~Phosphate~~
   - ~~NaF~~
   - ~~Detergent (Tween-20, Tween-80)~~



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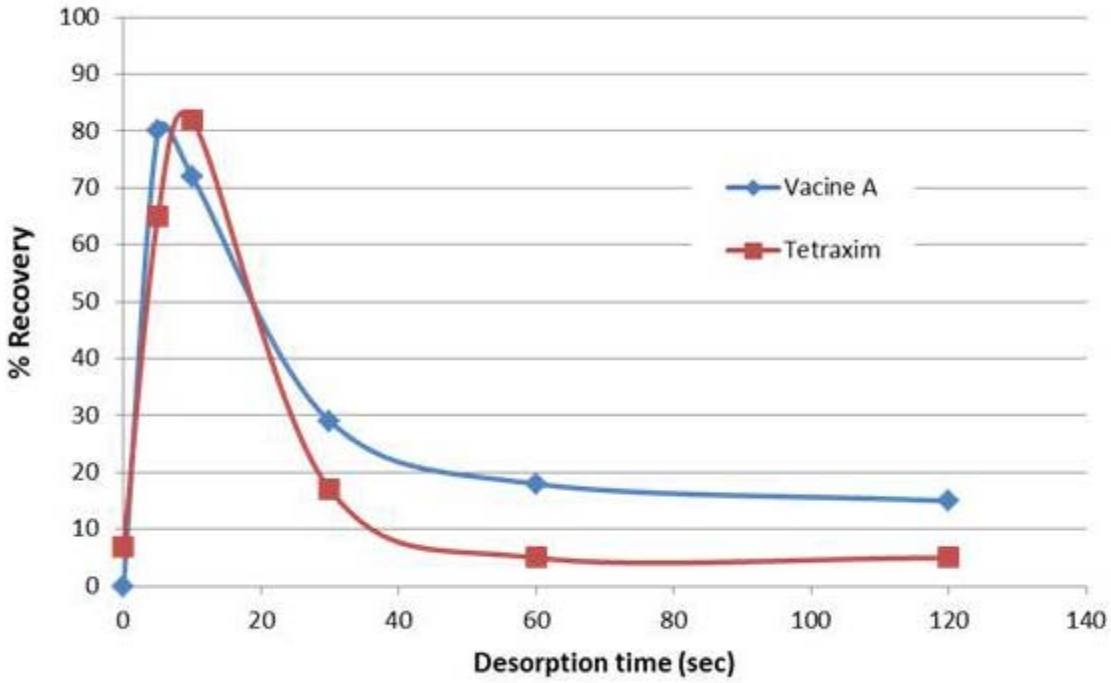
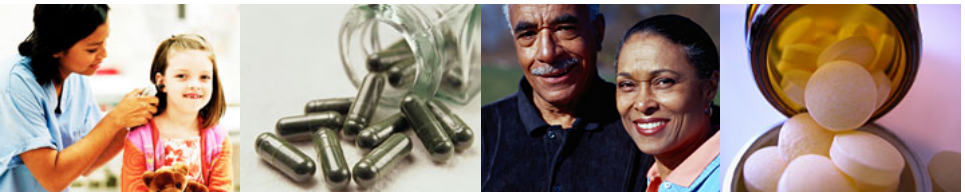
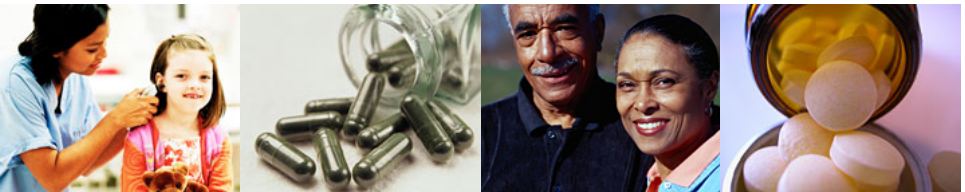
Rapid desorption with NaOH / Citrate neutralization



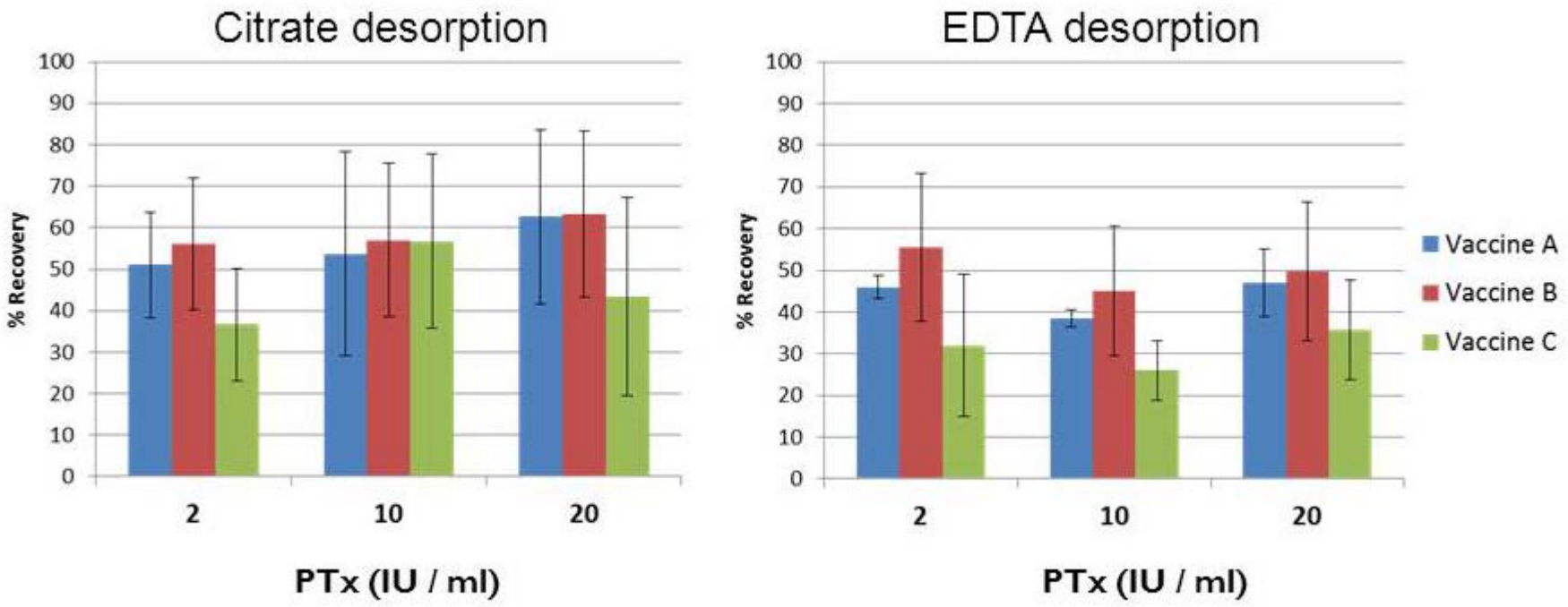


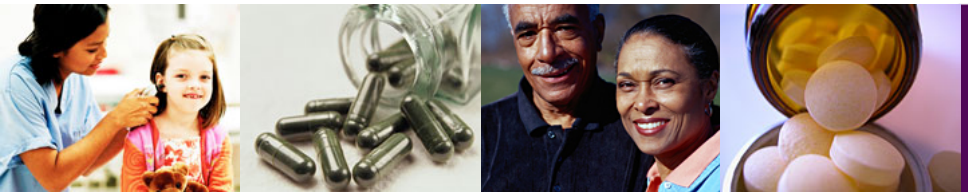
Desorption methods

1. Competition for adjuvant
   - ✗ Protein/peptide fragments
2. Dissolution of adjuvant
   - Citrate
   - ? NaOH (and neutralization with citrate)
3. Dissociation from adjuvant
   - EDTA
   - ✗ Phosphate
   - ✗ NaF
   - ✗ Detergent (Tween-20, Tween-80)



% Recovery of PTx spike from GSK vaccines





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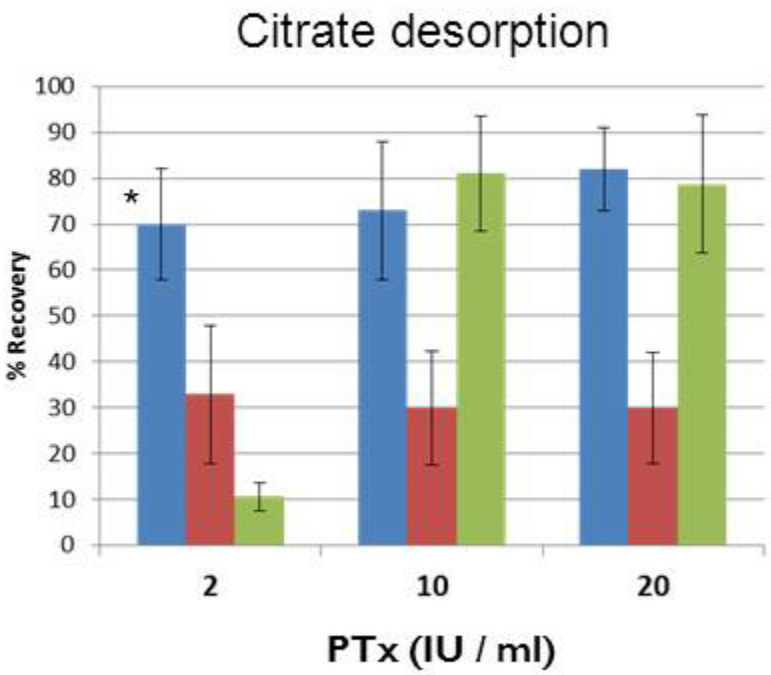
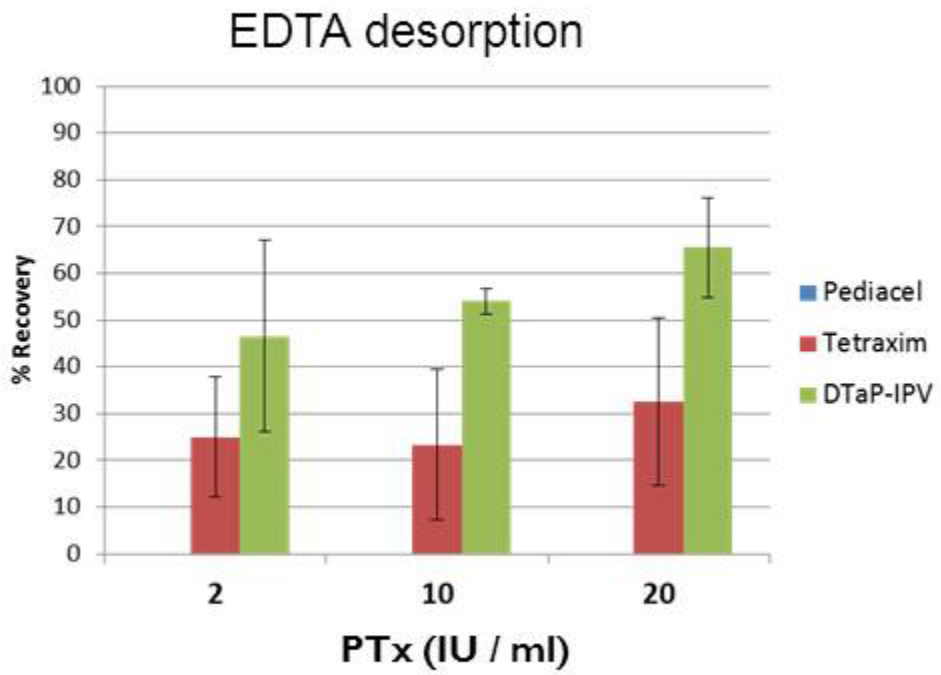
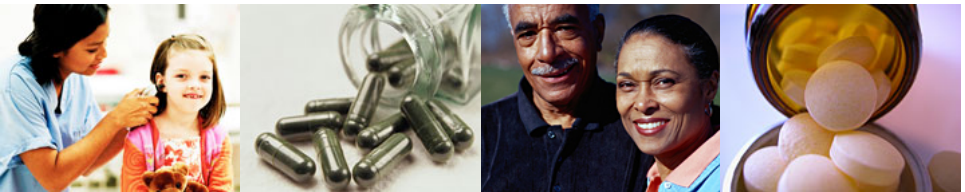
% Recovery of PTx spike from SP and SSI vaccines





(*Note: Pediacel not desorbed)



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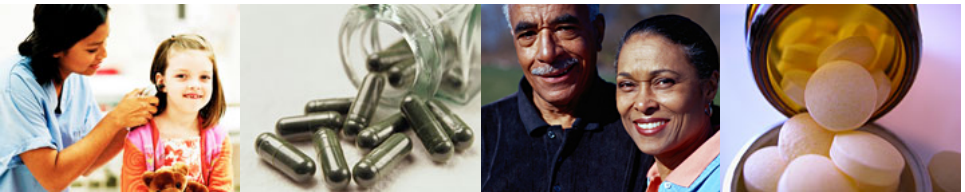
eHPLC Assay:
Detection of PTx in spiked aP vaccines



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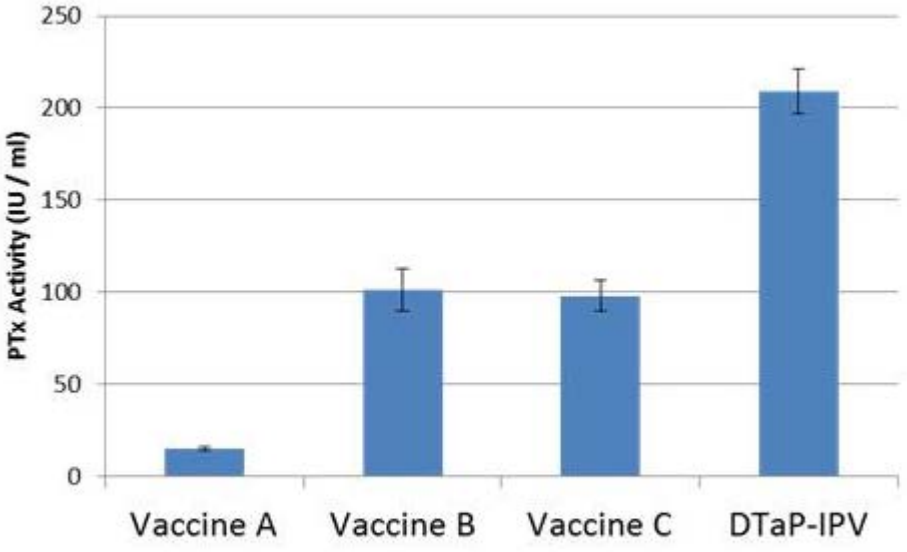
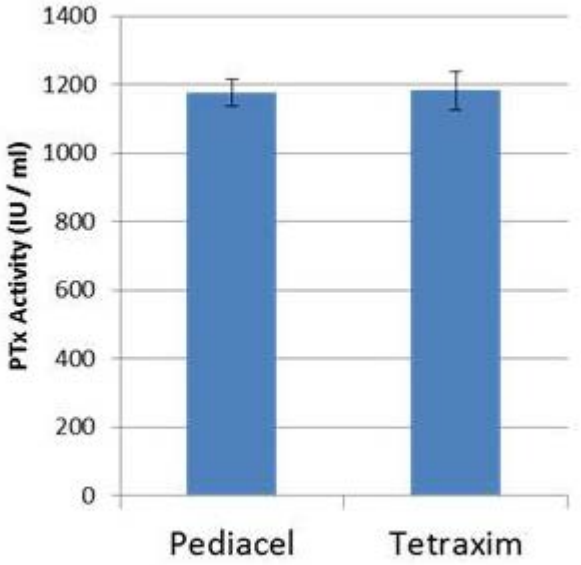
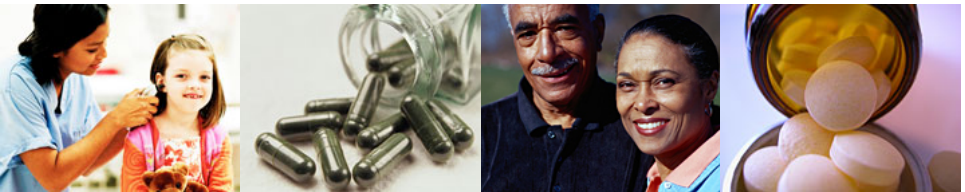
Background enzymatic activity of aP vaccines (eHPLC activity)







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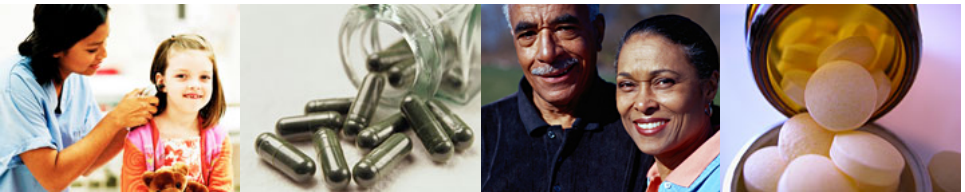
In vitro detection of PTx in spiked vaccines

Summary:

- PTx showed strong adsorption to vaccines adjuvented with $Al(OH)_3$
- Citrate and EDTA buffers provide good recovery of PTx spike from vaccines
- PTx added to vaccines can be detected at 2 IU/ml in fetuin binding and modified CHO clustering assays
- eHPLC may be able to detect PTx spike in some vaccines



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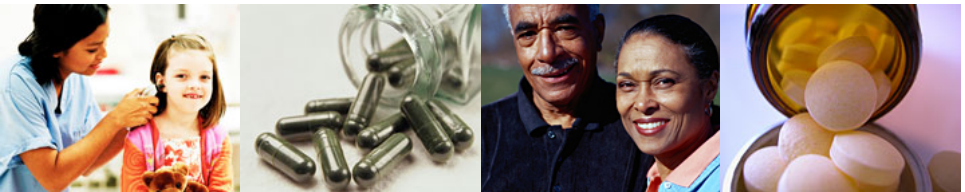
In vitro detection of PTx in spiked vaccines

Summary:

- Combined, the biochemical and cell based methods may provide a measure of residual PTx activity in vaccines with a sensitivity similar to that of the mouse HIST.
- In vitro assay methods need to be adapted/modified for each vaccine (eg: desorption method)



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Acknowledgements

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Dean Smith, PhD




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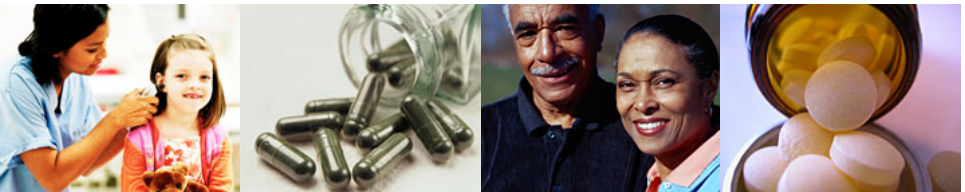
Thank you.



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