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

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CHO Cell Clustering Assay

Control (no PTx)

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

+ PTx (48 hrs)

+++
# CHO Cell Clustering Assay

Ph. Eur. Pertussis Toxin (BRP batch 1)

<table>
<thead>
<tr>
<th>Observer</th>
<th>PTx Conc. (IU/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>
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

Vaccine (1:8 – 1:32 dilution)

CHO Cells

Cell culture insert (porous)

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
## Modified CHO Cell Clustering Assay

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>0</th>
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<th>2</th>
<th>5</th>
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<tbody>
<tr>
<td>Vac. A</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Vac. B</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td></td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Vac. C</td>
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<tr>
<td></td>
<td>+/-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
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## Modified CHO Cell Clustering Assay

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Pediacel</td>
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<td>+++</td>
<td>+++</td>
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<tr>
<td></td>
<td>+/-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Tetraxim</td>
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<td>DTaP-IPV</td>
<td>-</td>
<td>++</td>
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<td>+++</td>
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</tbody>
</table>
Modified CHO Cell Clustering Assay

Summary:

• CHO cell clustering assay can be done on adjuvanted 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

![Diagram of Fetuin Binding Assay]

LOQ (in PBS) = 0.2 – 0.4 IU/ml
Fetuin Binding Assay – preparation of samples

1. Add Vaccine + PTx and Centrifuge
2. Remove supernatant and Centrifuge
3. Incubate 1 hr
4. Resuspend pellet in desorption buffer, incubate overnight
5. Collect supernatant, test in binding assay
Recovery of PTx by desorption of spiked vaccines

![Graph showing the recovery of PTx by desorption of spiked vaccines. The x-axis represents PTx (IU/mL) ranging from 0.1 to 100, and the y-axis represents absorbance ranging from 0 to 4.5. The graph includes a legend for different spiked vaccines: EDQM PTx, Vaccine A, Vaccine B, Vaccine C, Pediace, Tetraisim, and DTaP-IPV.](image)
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)
Rapid desorption with NaOH / Citrate neutralization
Desorption methods

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% Recovery of PTx spike from GSK vaccines

Citrate desorption

EDTA desorption

% Recovery

PTx (IU / ml)

Vaccine A
Vaccine B
Vaccine C
% Recovery of PTx spike from SP and SSI vaccines

Citrate desorption

EDTA desorption

(*Note: Pediacel not desorbed)
eHPLC Assay:
Detection of PTx in spiked aP vaccines
Background enzymatic activity of aP vaccines (eHPLC activity)
In vitro detection of PTx in spiked vaccines

Summary:

• PTx showed strong adsorption to vaccines adjuvanted with $\text{Al(OH)}_3$
• 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
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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