Cell Based Assays for Detection of Pertussis Toxin in Acellular Vaccines: The cAMP-PTx Assay and the Pertussis ATP Test (PAT)
BSP114 Study Phase 1
## Cell-based assays

<table>
<thead>
<tr>
<th></th>
<th>NVI</th>
<th>PEI</th>
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<tbody>
<tr>
<td><strong>Name</strong></td>
<td>cAMP-PTx assay</td>
<td>Pertussis ATP Test</td>
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<tr>
<td><strong>Mechanism</strong></td>
<td>Utilising PTx interferences in the signal transduction pathway</td>
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<td><strong>Cells type</strong></td>
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<td>cAMP</td>
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Assay description

Gs coupled β₂-adrenoceptor

Gi coupled α₂-adrenoceptor

Adenylate cyclase

ATP → cAMP

ADP-Ribose

PTx

PEI

NVI
Pertussis ATP Test (PAT)

Luciferin + ATP + O₂ → Oxyluciferin + AMP + PP₁ + CO₂ + Light

GDP

Gs coupled β₂-adrenoceptor

α₅

GTP

Gi coupled α₂-adrenoceptor

α₅ + Adenylate cyclase

ATP → cAMP

Luciferase

Mg²⁺
Overview: Pertussis-ATP-Test (PAT)

1. Cell preparing
   - Cryo-PBMCs

2. Incubation
   - Cells + samples (spiked vaccines, PTx)
   - 37°C, 5% CO₂ for 3h

3. Measurement
   - Determination ATP content
   - Measuring via luminometer

Cryo-PBMCs: cryoconserved PBMCs; PBMCs: Peripheral blood mononuclear cells.
# Schedule of PAT/BPS114

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<tr>
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<th>Adjuvant</th>
<th>BRP1-PTx spike [IU/ml]</th>
<th>Desorption</th>
<th>Repetitions</th>
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<tr>
<td>1</td>
<td>GSK sample A</td>
<td>Al(OH)3</td>
<td>80, 40, 20, 10, 2 and 0</td>
<td>no</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Sanofi Pasteur Canada-Pediaacel</td>
<td>AlPO4</td>
<td>80, 40, 20, 10, 2 and 0</td>
<td>no</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>SSI-DTaP-IPV</td>
<td>Al(OH)3</td>
<td>80, 40, 20, 10, 2 and 0</td>
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Preliminary result PAT

- Adsorbed vaccines spiked with Pertussis Toxin
- $2 \times 10^5$ Cryo-PBMCs; pool of 4 donors
Results BSP114 study: PAT

- Example one experiment (out of three)
- $5 \times 10^5$ Cryo-PBMCs; pool of 6 donors
PAT: Outcome/conclusion

• Easy performable, fast and cheap, but not able to reach the given sensitivity range

→ In the future our focus will be on the cAMP-PTx assay
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Assay description
Study design 1
Study design: two ELISA kits

Enzo
1. Goat-anti-rabbit IgG
2. cAMP-alkaline phosphatase
3. pNpp substrate (405 nm)
4. Standard 200 pmol/mL-0.78 pmol/mL cAMP

GE Healthcare
1. Donkey-anti-rabbit IgG
2. cAMP-HRP
3. TMB substrate (450 nm)
4. Standard 16 pmol/mL-0.12 pmol/mL cAMP
Study design 2

Sensitivity

Solvent PTx

Spiking time

PTx standard

Experiments (BSP114)

Lactate dehydrogenase
Calculation of results
Solvents (see below)
Spiking time
# Schedule of cAMP-PTx assay/BPS114

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Results NVI: Experiment C (selected)

PTx spiked vaccines (ratio)
Experiment C

Ratio (PTx spiked vaccines/unsplited vaccines)

- GSK Sample A Al(OH)3
- Sanofi DTaP-Hib-IPV AlPO4
- SSI aP Al(OH)3
- SSI DTaP-IPV Al(OH)3
- PTx in PBS

A10 cells [5000 cells/well]
Method transfer: Results PEI Experiment 2 (selected)
PTx-cAMP assay

Conclusions:
1. Method transfer successful
2. Sensitivity 20/10 IU

Pros:
- No need for desorption
- Functional assay
- Can measure activity A and B combined

Cons:
- Variations PTx standard, vaccines
- Problems with ELISA kits
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