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

GSK/Sanofi/SSI
↓
EDQM

[Images of boxes and trays with vaccine vials]
## Cell-based assays

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

Gs coupled β2-adrenoceptor

Gi coupled α2-adrenoceptor

Adenylate cyclase +

GTP → cAMP

ATP → PEI

NVI

ADP-Ribose

PTx
Pertussis ATP Test (PAT)

Luciferin + ATP + O₂ → Luciferase → Oxyluciferin + AMP + PP₁ + CO₂ + Light
Overview: Pertussis-ATP-Test (PAT)

1. Cell preparing

2. Incubation

3. Measurement

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

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Adjuvant</th>
<th>BRP1-PTx spike [IU/ml]</th>
<th>Desorption</th>
<th>Repetitions</th>
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<tr>
<td>GSK sample A</td>
<td>Al(OH)3</td>
<td>80, 40, 20, 10, 2 and 0</td>
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</tr>
<tr>
<td>Sanofi Pasteur Canada-Pediaacel</td>
<td>AlPO4</td>
<td>80, 40, 20, 10, 2 and 0</td>
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<tr>
<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

![Graph showing ATP content after 1 h incubation with different concentrations of Pertussis Toxin (PTx) and NaCl.](chart.png)
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
# Cell-based assays

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Assay description
Study design 1

Protocol
- 96 well
- PTx/Vaccine
- IBMX
- + Isoprenaline
- Lysis
- ELISA

Mechanism
- ATP
- Adenylate cyclase
- cAMP
- PFE
- AMP
- $G_s$
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
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Results NVI: Experiment C (selected)

PTx spiked vaccines (ratio)

Experiment C

Ratio (PTx spiked vaccines/unsptiked 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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