Cell Based Assays for Detection of Pertussis Toxin in Acellular Vaccines: The cAMP-PTx Assay and the Pertussis ATP Test (PAT)

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BSP114 Study Phase 1

GSK/Sanofi/SSI

EDQM

Earth

BSP-044
Fire & Vaccines
# Cell-based assays

<table>
<thead>
<tr>
<th>Name</th>
<th>NVI</th>
<th>PEI</th>
</tr>
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<tbody>
<tr>
<td><strong>Mechanism</strong></td>
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<td><strong>Cells type</strong></td>
<td>Vascular smooth muscle cell line A10</td>
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<td>cAMP</td>
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- **Name**: cAMP-PTx assay, Pertussis ATP Test
- **Mechanism**: Utilising PTx interferences in the signal transduction pathway
- **Cells type**: Vascular smooth muscle cell line A10, Peripheral blood mononuclear cells (PBMCs)
- **Measured**: cAMP, ATP
Assay description
Pertussis ATP Test (PAT)

\[
\text{Luciferin} + \text{ATP} + \text{O}_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + \text{PP}_1 + \text{CO}_2 + \text{Light}
\]
Overview: Pertussis-ATP-Test (PAT)

1. Cell preparing
   - Cryo-PBMCs

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

3. Measurement
   - Determination ATP content
     - Measuring via luminometer

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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</thead>
<tbody>
<tr>
<td>1 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 Sanofi Pasteur</td>
<td>AlPO4</td>
<td>80, 40, 20, 10, 2 and 0</td>
<td>no</td>
<td>3</td>
</tr>
<tr>
<td>3 SSI-DTaP-IPV</td>
<td>Al(OH)3</td>
<td>80, 40, 20, 10, 2 and 0</td>
<td>no</td>
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</tr>
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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

![Bar chart showing ATP content after 3 h incubation with different PTx concentrations for various vaccine formulations: GSK, Sanofi Pasteur, SSI DTaP-IPV, and PTx in PBS. The x-axis represents the number of cells per ml ($5 \times 10^5$ Cells/ml), and the y-axis shows the RLU (relative light units). The chart includes error bars indicating variability.]
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
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

1

2

3
Solvents (see below)
Spiking time

[Diagram showing bar graph with different categories and data points, indicating some form of comparison or analysis.]
### Schedule of cAMP-PTx assay/BPS114

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Results NVI: Experiment C (selected)

PTx spiked vaccines (ratio)

Experiment C

- [20 IU/mL] vaccine/medium [6.7 IU/mL] final
- [10 IU/mL] vaccine/medium [3.3 IU/mL] final
- [2 IU/mL] vaccine/medium [0.7 IU/mL] final
- [0 IU/mL] vaccine/medium [0 IU/mL] final

Ratio (PTx spiked vaccines/unspeckled 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
Acknowledgement

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