

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



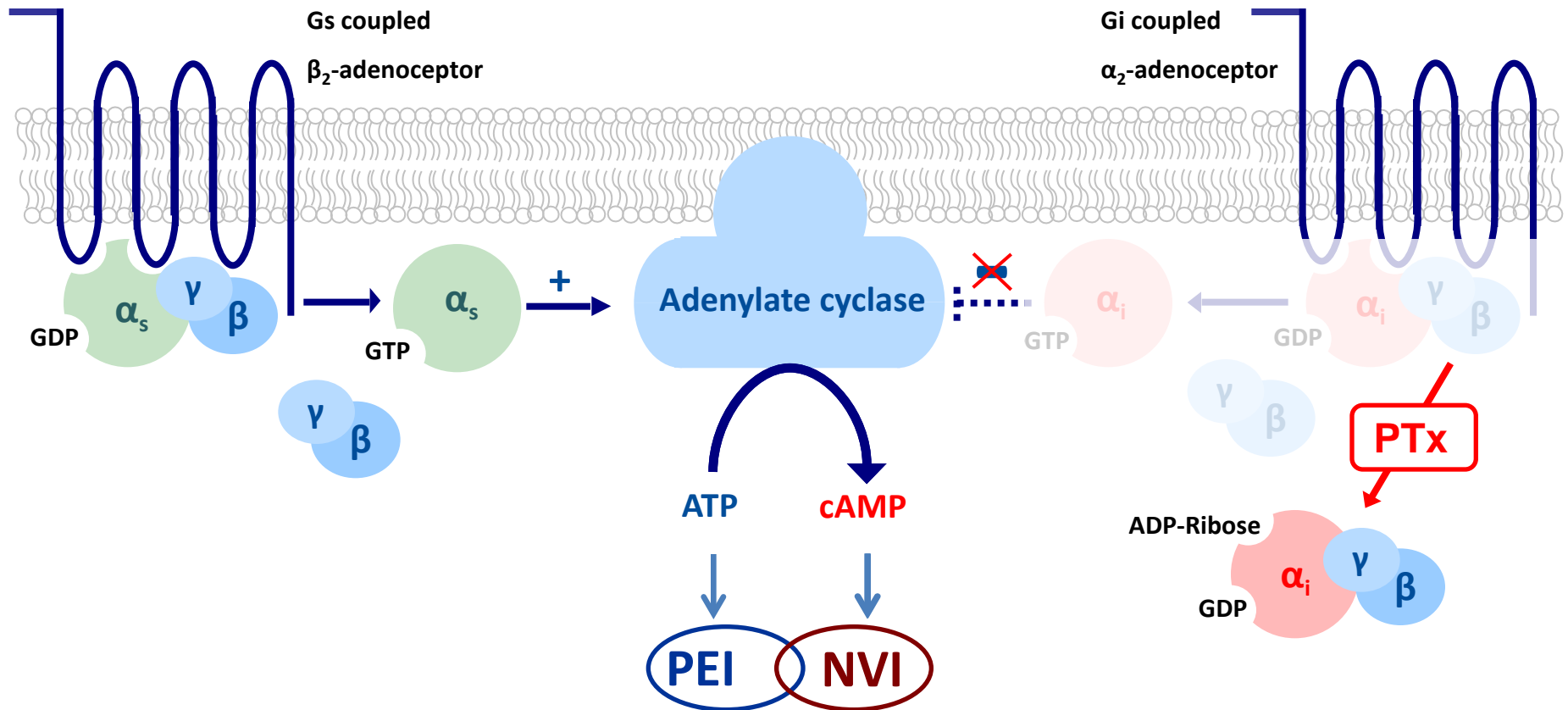
EDQM



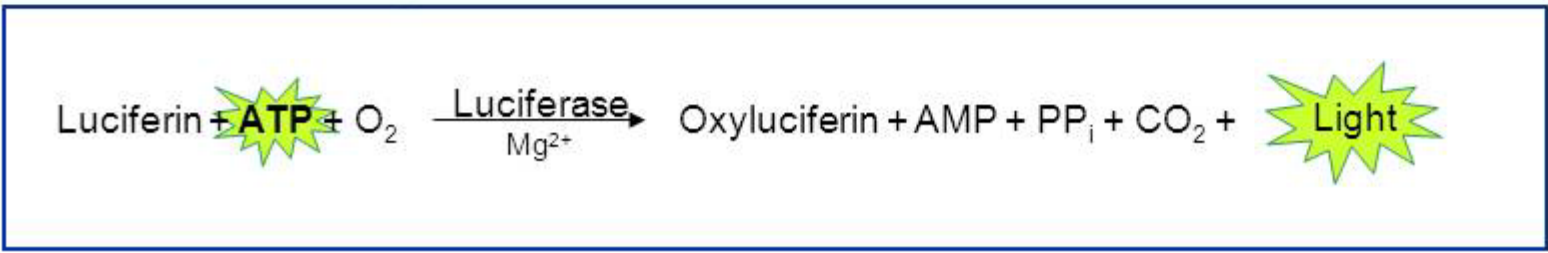
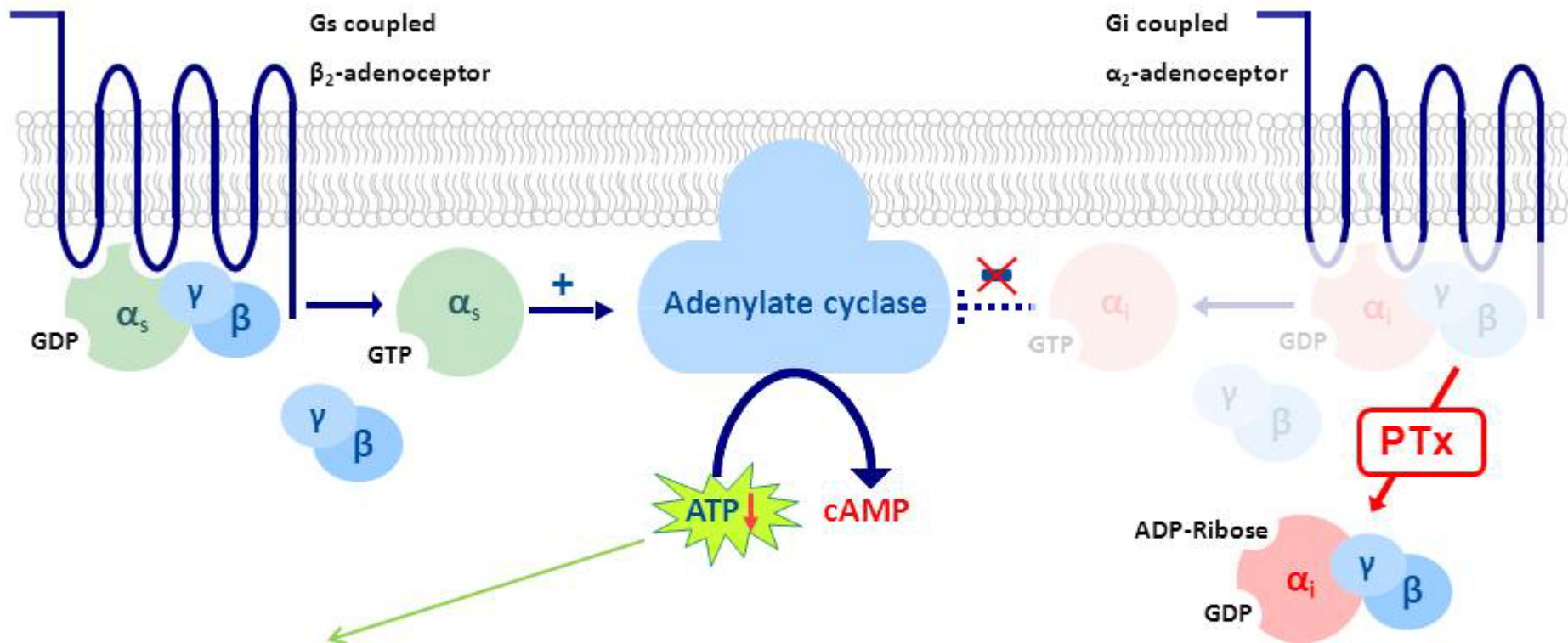
Cell-based assays

	NVI	PEI
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

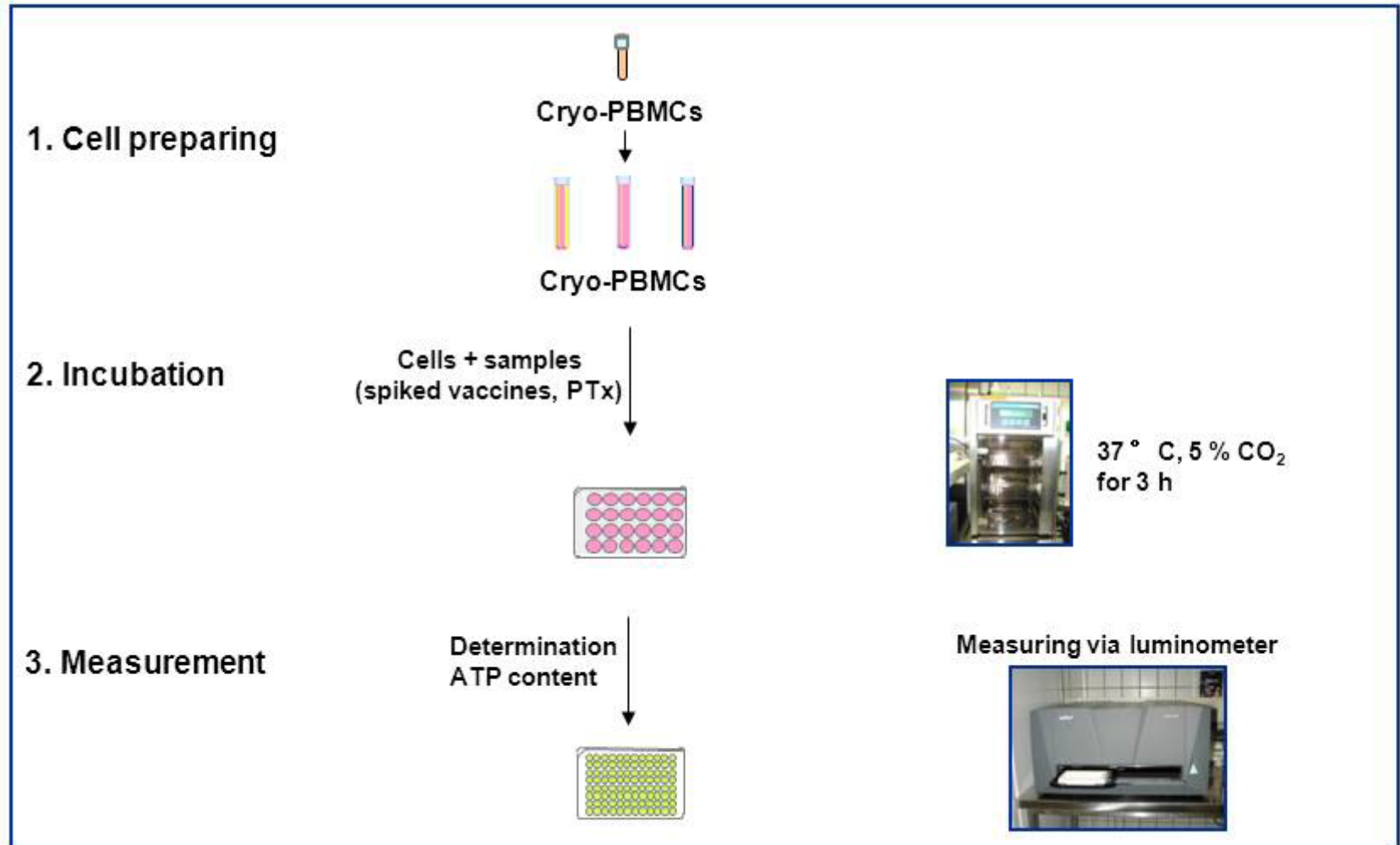
Assay description



Pertussis ATP Test (PAT)



Overview: Pertussis-ATP-Test (PAT)



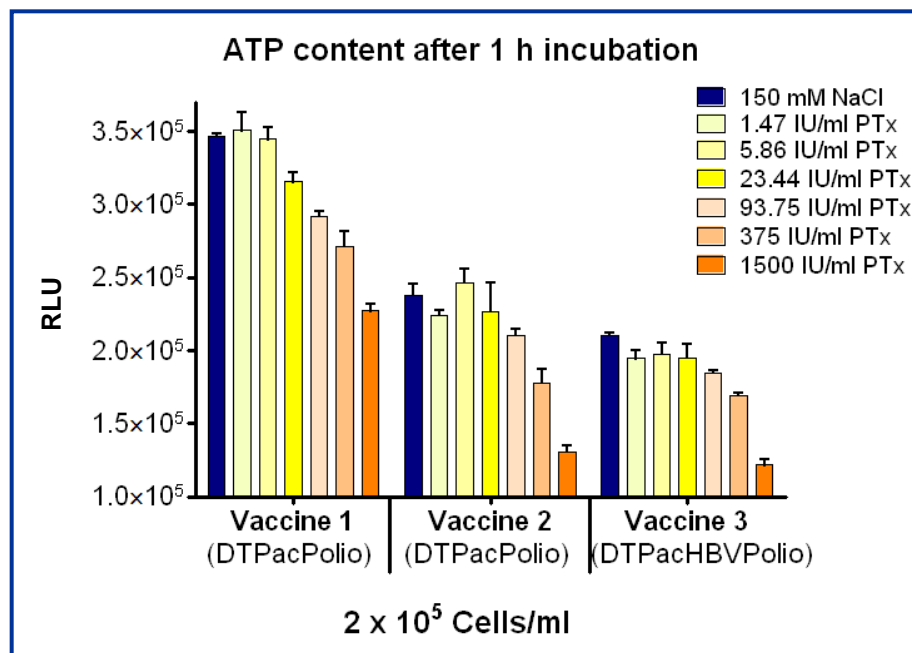
Cryo-PBMCs: cryoconserved PBMCs; PBMCs: Peripheral blood mononuclear cells.

Schedule of PAT/BPS114

	Vaccines	Adjuvant	BRP1-PTx spike [IU/ml]	Desorption	Repetitions
1	GSK sample A	Al(OH) ₃	80, 40, 20, 10, 2 and 0	no	3
2	Sanofi Pasteur Canada-Pediacel	AlPO ₄	80, 40, 20, 10, 2 and 0	no	3
3	SSI-DTaP-IPV	Al(OH) ₃	80, 40, 20, 10, 2 and 0	no	3

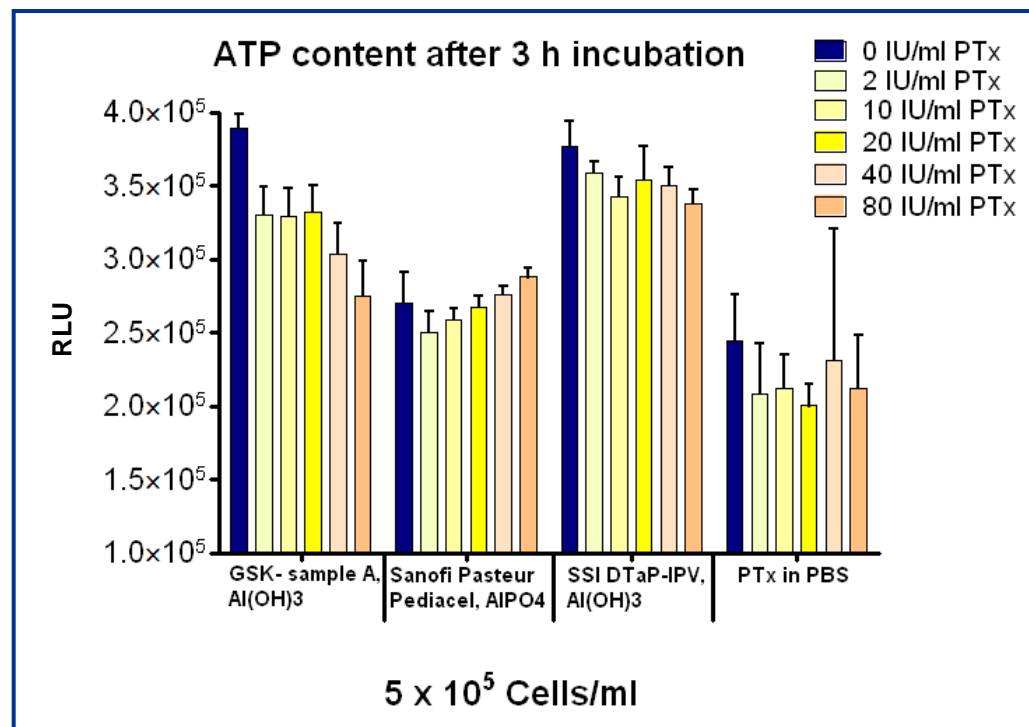
Preliminary result PAT

- Adsorbed vaccines spiked with Pertussis Toxin
- 2×10^5 Cryo-PBMCs; pool of 4 donors



Results BSP114 study: PAT

- Example one experiment (out of three)
- 5×10^5 Cryo-PBMCs; pool of 6 donors



PAT: Outcome/conclusion

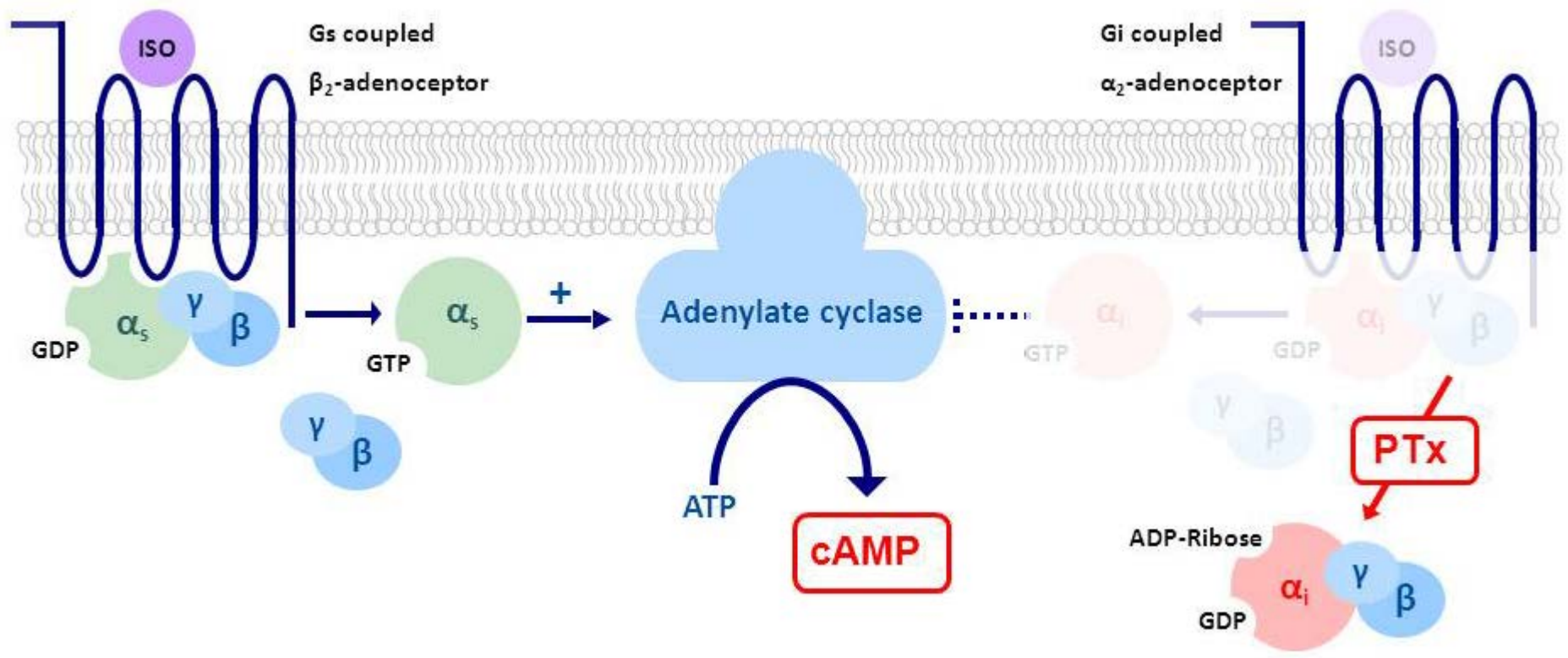
- Easy performable, fast and cheap, **but** not able to reach the given sensitivity range

→ In the future our focus will be on the
cAMP-PTx assay

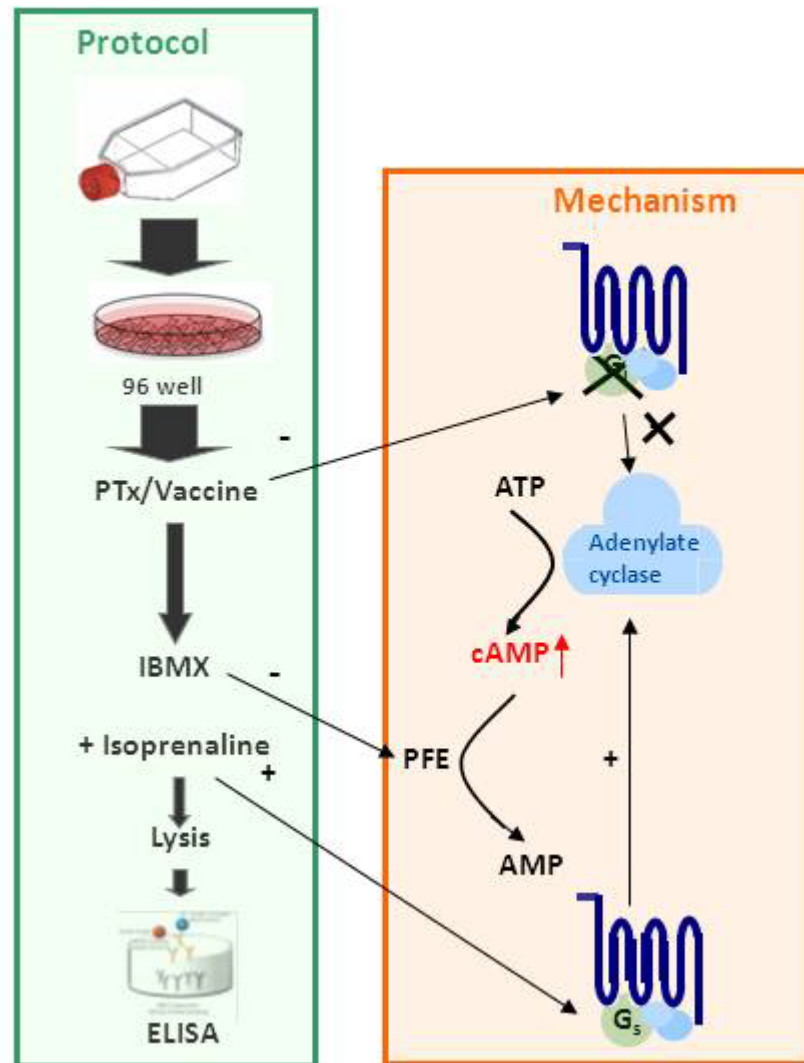
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Measured	cAMP	ATP

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

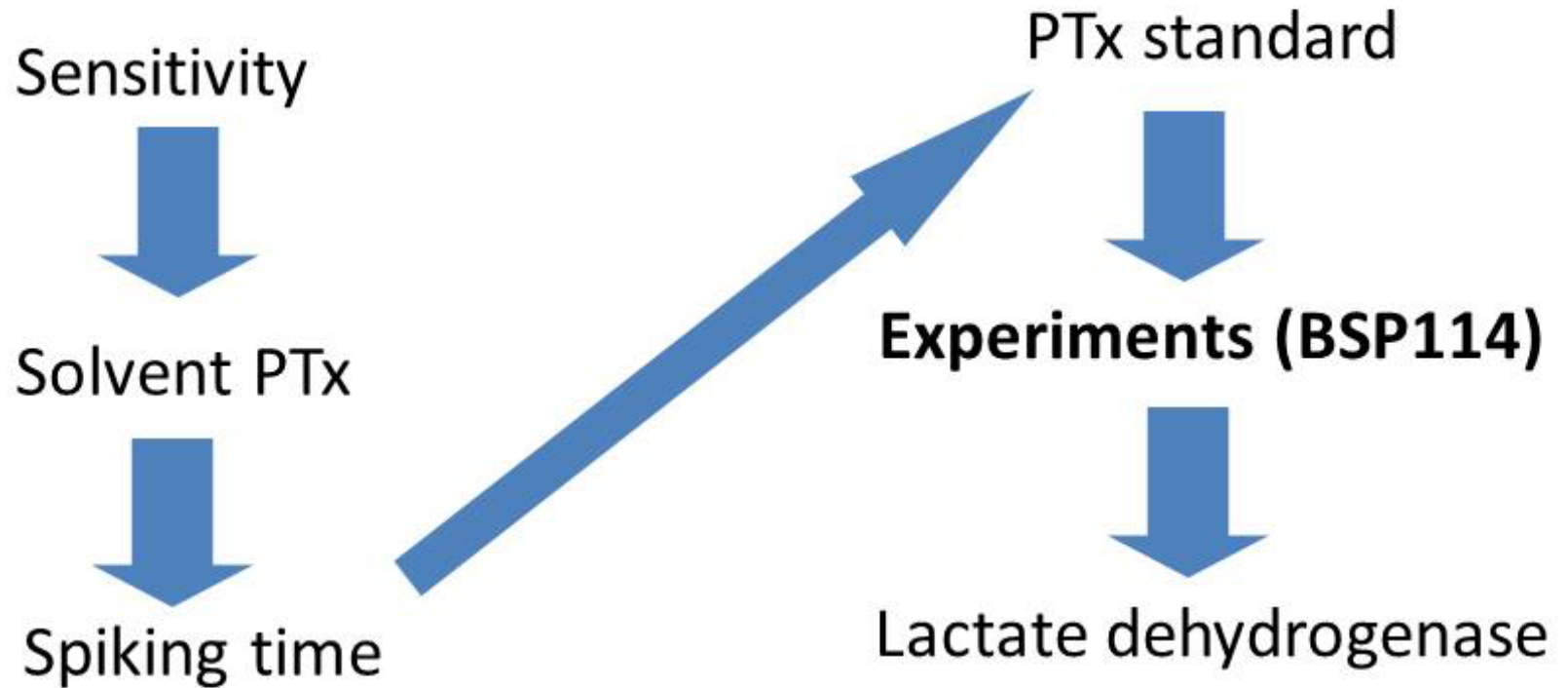


GE Healthcare

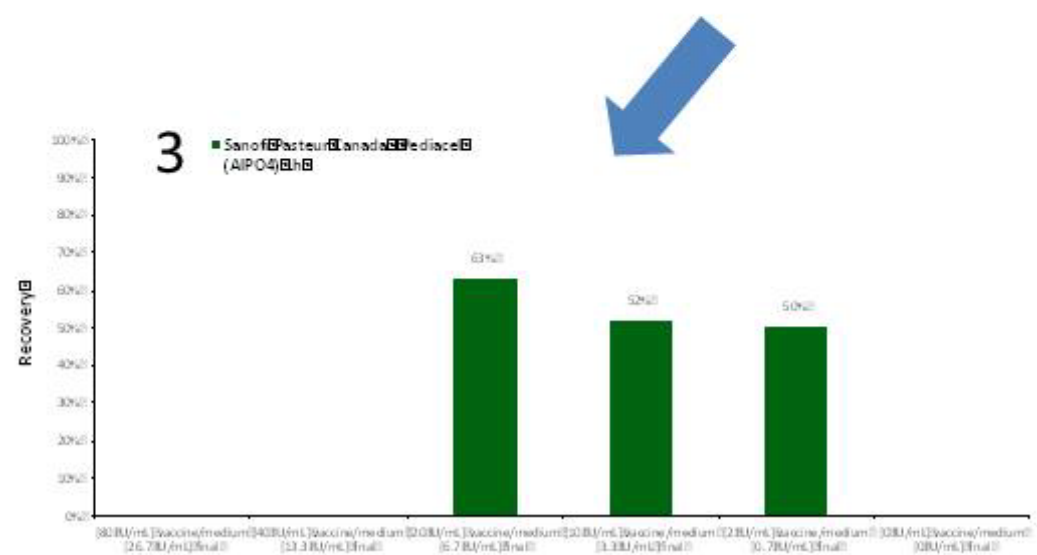
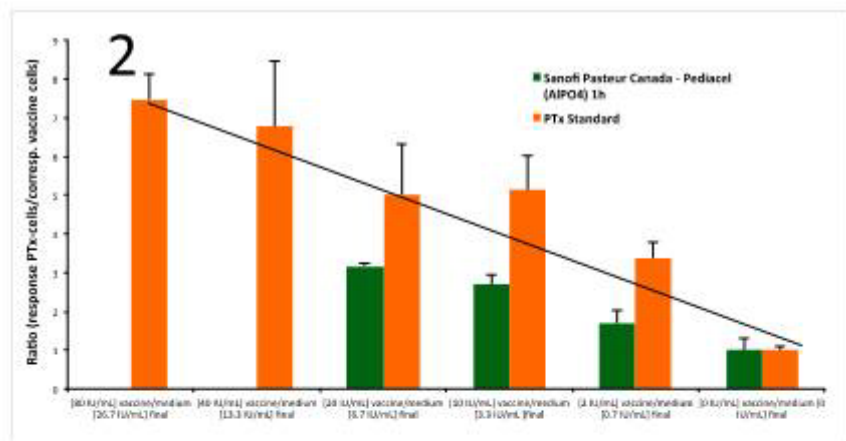
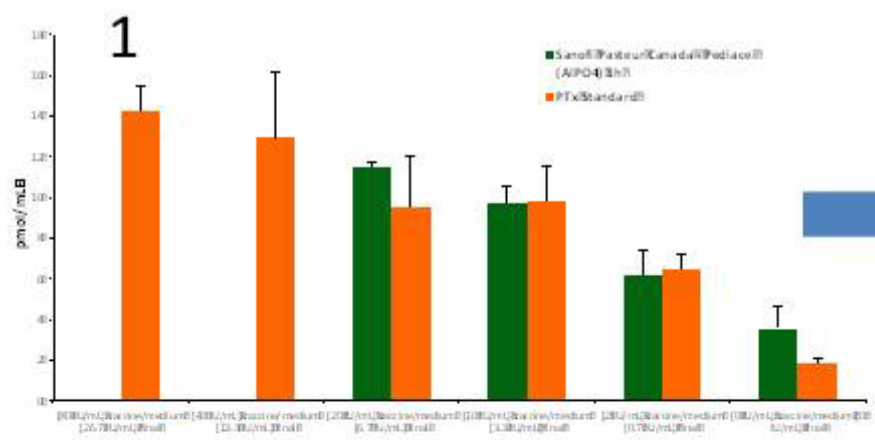
GE Healthcare
GE Healthcare

1. Donkey-anti-rabbit IgG
Donkey-anti-rabbit IgG
2. cAMP-HRP
cAMP-HRP
3. TWB substrate (450 nm)
TWB substrate (450 nm)
4. Standard 16 pmol/mL-0.12 pmol/mL cAMP
Standard 16 pmol/mL-0.12 pmol/mL cAMP

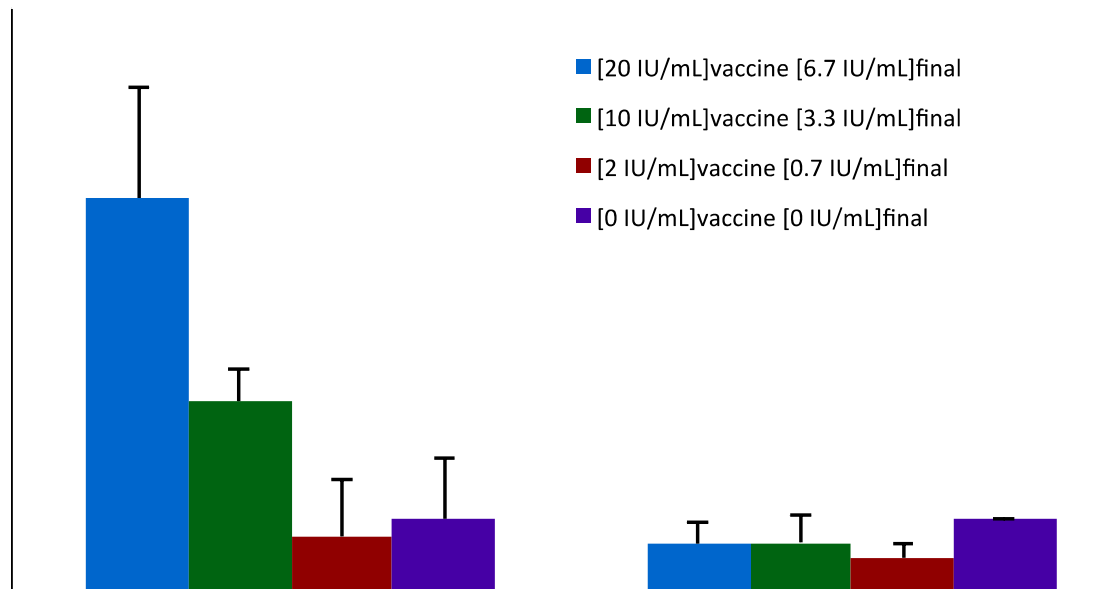
Study design 2



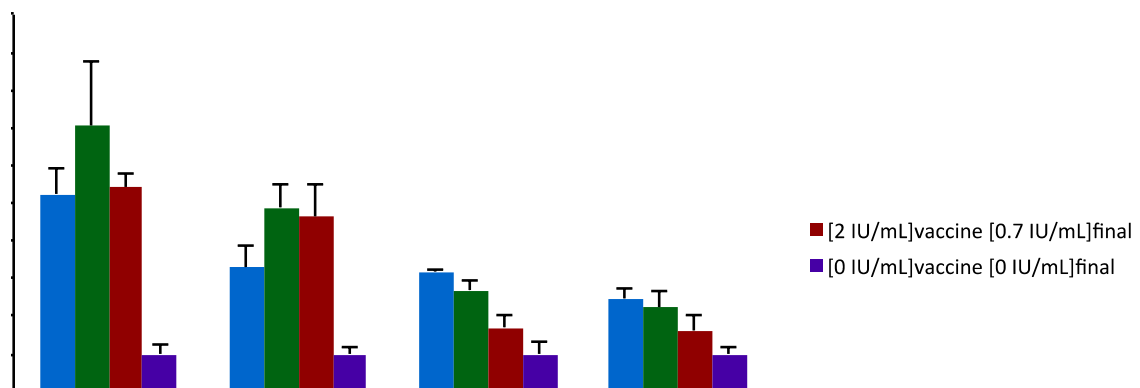
Calculation of results



Solvents (see below)



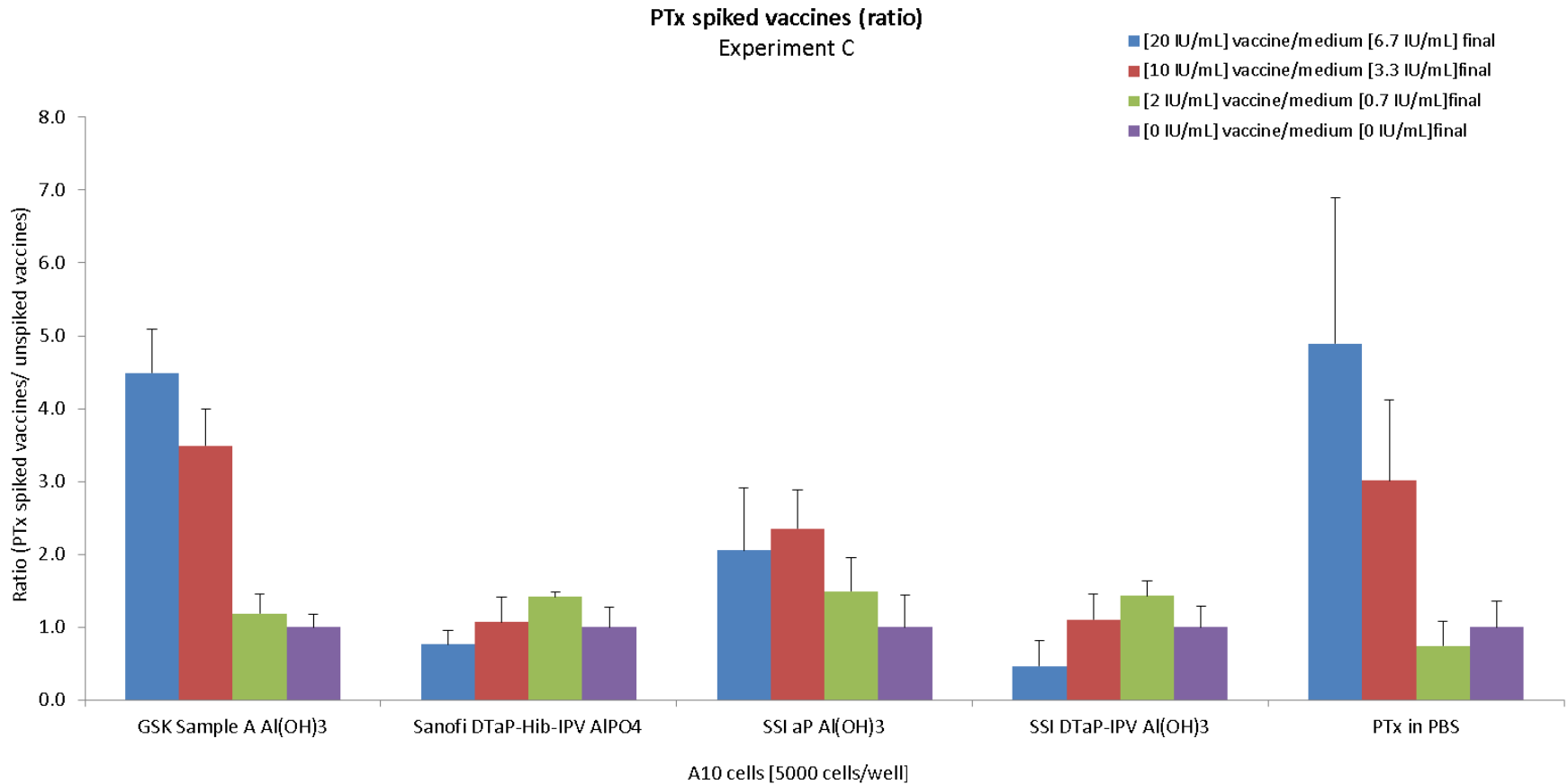
Spiking time



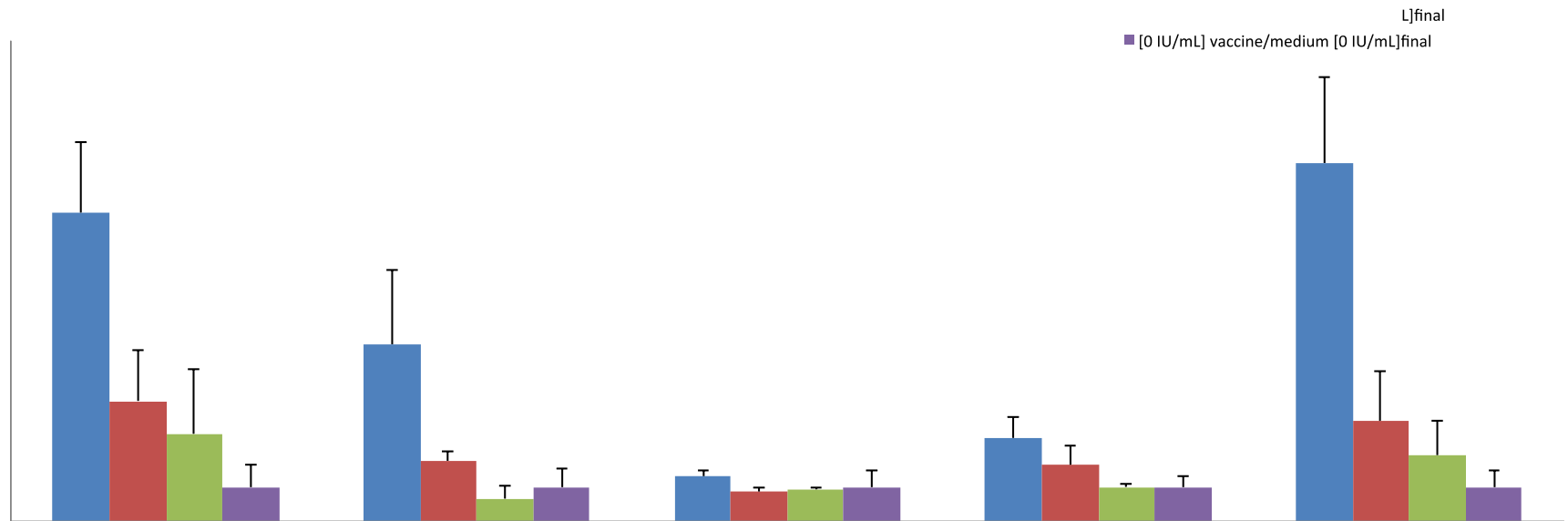
Schedule of cAMP-PTx assay/BPS114

	Vaccines	Adjuvant	BRP1-PTx spike [IU/ml]	Desorption	Repetitions	
					NVI	PEI
1	GSK sample A	Al(OH) ₃	20, 10, 2 and 0	no	5	3
2	Sanofi Pasteur Canada-Pediacel	AlPO ₄	20, 10, 2 and 0	no	5	3
3	SSI-Toxoid	Al(OH) ₃	20, 10, 2 and 0	no	3	3
4	SSI-DTaP-IPV	Al(OH) ₃	20, 10, 2 and 0	no	3	3

Results NVI: Experiment C (selected)



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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