Posterprijs voor ontwikkeling *in vitro* veiligheidstest kinkhoestvaccin

Tijdens de DAS meeting is de poster van onderzoekers van Hogeschool Utrecht en RIVM beloond met een posterprijs. De poster beschrijft een nieuwe benadering voor de ontwikkeling van een *in vitro* assay voor kinkhoest (pertussis) vaccin veiligheidsonderzoek.

De regelgeving vereist tests voor het bepalen van vaccinveiligheid. Voor de batchcontrole van kinkhoestvaccin is de 'histamine sensitisation test' (HIST) in muizen momenteel de standaard testmethode. Het mechanisme achter de HIST is echter grotendeels onbekend, waardoor getwijfeld kan worden aan de klinische relevantie van deze test. Bovendien is bij HIST de dood van de muis het eindpunt en wordt dus zowel uit oogpunt van dierenwelzijn als standaardisatie sterk gepleit voor vervanging van deze dierproef door een *in vitro* alternatief.

Het project dat wordt beschreven in de prijswinnende poster is gericht op de ontwikkeling van een mechanistisch testassay op basis van humane cellen voor het veiligheidsonderzoek van acellulair kinkhoestvaccin. Via micro array analyse wordt gezocht naar pathways en mogelijke biomarkers. Enkele gevonden pathways kunnen al gelinkt worden aan klinische verschijnselen. Uit deze pathways worden genen geselecteerd voor de ontwikkeling van een *in vitro* testmethode voor vaccinveiligheid, die uiteindelijk de huidige *in vivo* test zou moeten vervangen.

Op de volgende pagina vindt u de poster.

University of Applied Sciences
Research Centre Technology & Innovation

Rijksinstituut voor Volksgezondheid en Milieu

An innovative approach to develop an *in vitro* assay for pertussis vaccine safety testing

S.F.C. Vaessen[1], R.J. Vandebriel[2], M.W.P. Bruysters[2], S. Verkoeijen[1], J.L.A. Pennings[2], C.A.M. Krul[1] and A.M. Akkermans[2]

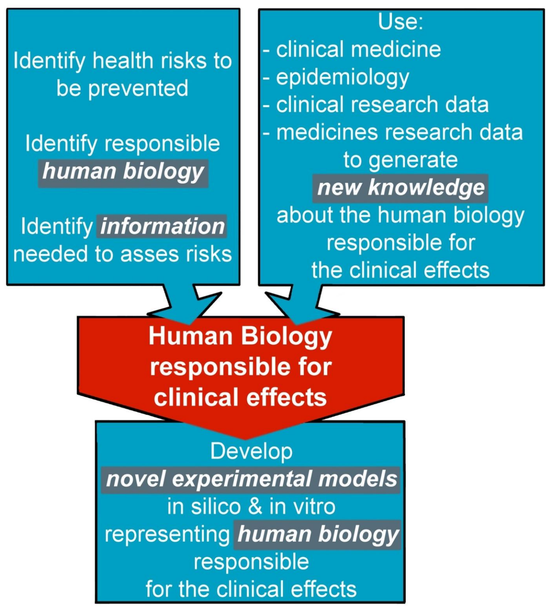
1) Innovative Testing in Life Sciences and Chemistry, University of Applied Sciences Utrecht, the Netherlands; *stefan.vaessen@hu.nl*
2) National Institute for Public Health and the Environment, Bilthoven, the Netherlands

Background

Pertussis toxin (PTx) is the major virulence factor of *Bordetella pertussis* and in detoxified form (PTd) an essential component of acellular pertussis vaccines. PTx can cause severe harmful effects therefore regulatory authorities require tests to demonstrate vaccine safety. The histamine sensitisation test (HIST) in mice is currently the standard method for batch release testing of pertussis vaccines. The exact mechanism underlying the HIST is largely unknown making its clinical relevance doubtful. Moreover it is al lethal test, making replacement a priority. With respect to animal welfare and considering standardisation, an *in vitro* test is highly preferable. The aim of our project is to develop a mechanism-based human cell assay for safety evaluation of acellular pertussis vaccines and eventually to replace the current *in vivo* test.

Approach

✓ Six different cell types of human origin are exposed to PTx for 2h.
✓ Microarray analysis to identify signalling pathways and potential biomarkers.
✓ Incubate MoDCs from 4 different donors with PTx, LPS, LOS and vehicle control to investigate sensitivity, specificity and reproducibility.
✓ PTx-responsive gene profile in whole blood (MoDCs), confirmation by ELISA.



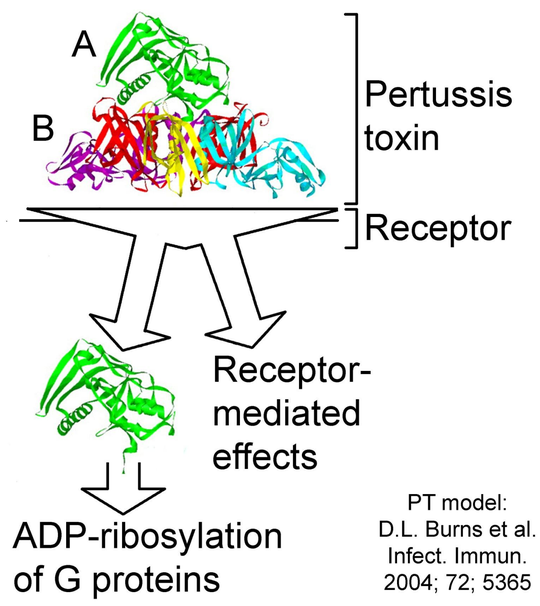
Adapted from: ASAT; reversing the paradigm in toxicity testing, B. Sangster, www.alttox.org

Assuring Safety without Animal Testing

Six different human cell types

Name	Description	Cell type
BEAS2B	Broncheal epithelial cells	Cell line
HMVEC	Cardiac microvascular endothelial cells	Primary cells
EA.Hy926	Hybrid of umbilical vein endothelial cells with epithelial cell line A549	Cell line
MRC5	Fetal lung fibroblast cells	Cell line
HPASMC	Pulmonary artery smooth muscle cells	Primary cells
MoDCs	Monocyte-derived dendritic cells	Primary cells

Two effector pathways of PTx



PT model: D.L. Burns et al. Infect. Immun. 2004; 72; 5365

Results

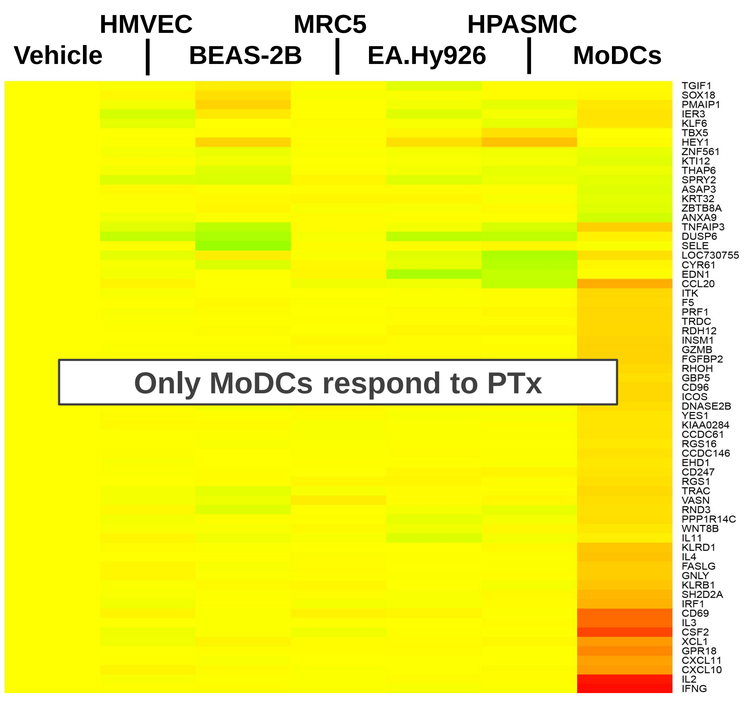


Figure 1. Results of RNA expression induced by PTx in 6 different human cell types. Primary MoDCs are differentiated from whole blood donations.

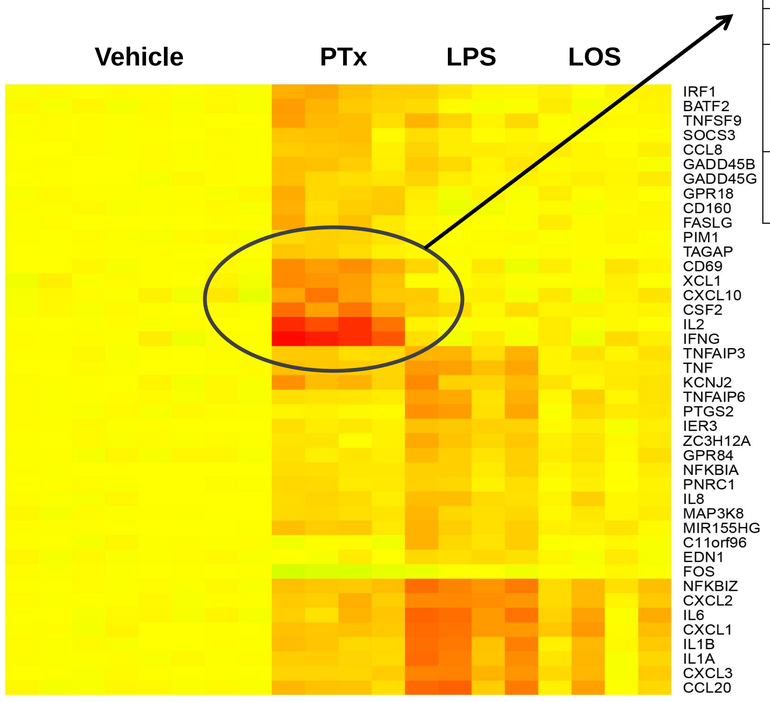


Figure 2. Results of RNA expression in MoDCs demonstrate a PTx-specific induction of 6 genes related to immune and inflammatory pathways in MoDCs.

Gene symbol	Gene name	GeneID	Vehicle	PTX	LPS	LOS	FDR
IFNG	interferon, gamma	3458	1	92.0	1.2	1.3	4,54E-07
IL2	interleukin 2	3558	1	50.3	1.0	1.1	2,03E-07
XCL1	chemokine (C motif) ligand 1	6375	1	7.5	1.1	1.1	5,5E-05
CD69	CD69 molecule	969	1	8.5	1.2	1.3	0,000116
CSF2	colony stimulating factor 2 (granulocyte-macrophage)	1437	1	11.1	1.9	1.3	0,000116
CXCL10	chemokine (C-X-C motif) ligand 10	3627	1	7.1	1.5	1.3	0,01251

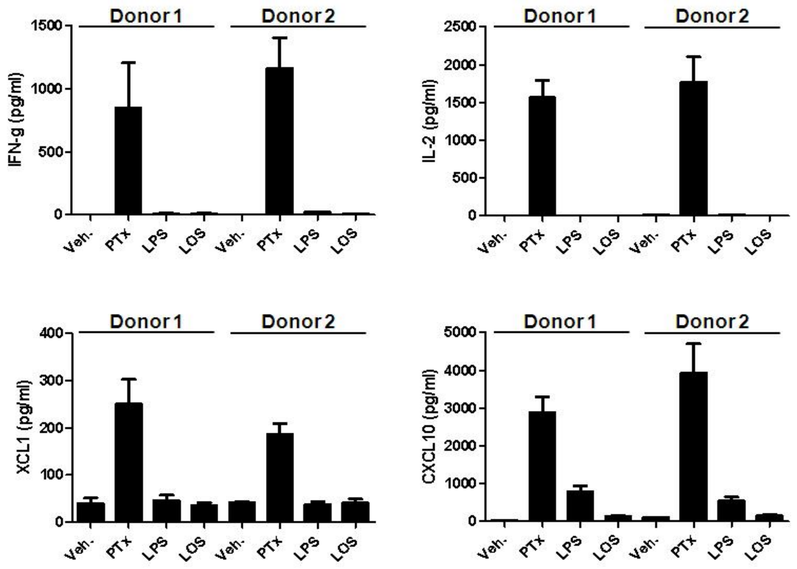


Figure 3 Confirmation of PTx-induced RNA expression at the protein level using ELISA

Conclusion

The proposed approach identified signal transduction pathways and some of them can be linked to the observed clinical effects of PTx. Genes obtained from these pathways are selected for the development of an *in vitro* method for the safety assessment of vaccines.

Further development towards a functional assay:
- Validate assay using whole vaccines in an international ring-trial (including pharmaceutical industry and regulators)
- Determine sensitivity of response (detection limit) and whether the presence of adjuvant in vaccines interferes with the assay
- Optimize differentiation and incubation of MoDCs
- Examine possibility to use reporter cell line instead of MoDCs to reduce variability

This study was funded by the National Institute for Public Health and the Environment (project S/360001).