Using the Monocyte Activation Test to determine the pyrogenicity of OMV preparations

Collaboration study Intravacc and Sanquin

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Whooping cough

- Highly contagious respiratory tract infection
- Caused by *Bordetella pertussis*
- Severe disease and even mortality in infants and young children – highest risks for infants too young to be vaccinated
- National immunisation programmes
  - Vaccination of infants: at least 3x during infancy and booster in children 1-6 years
  - Additional strategies
    - Booster in adolescents/adults
    - Booster in pregnant women to provide maternal antibodies to newborn infants

Pertussis Vaccines

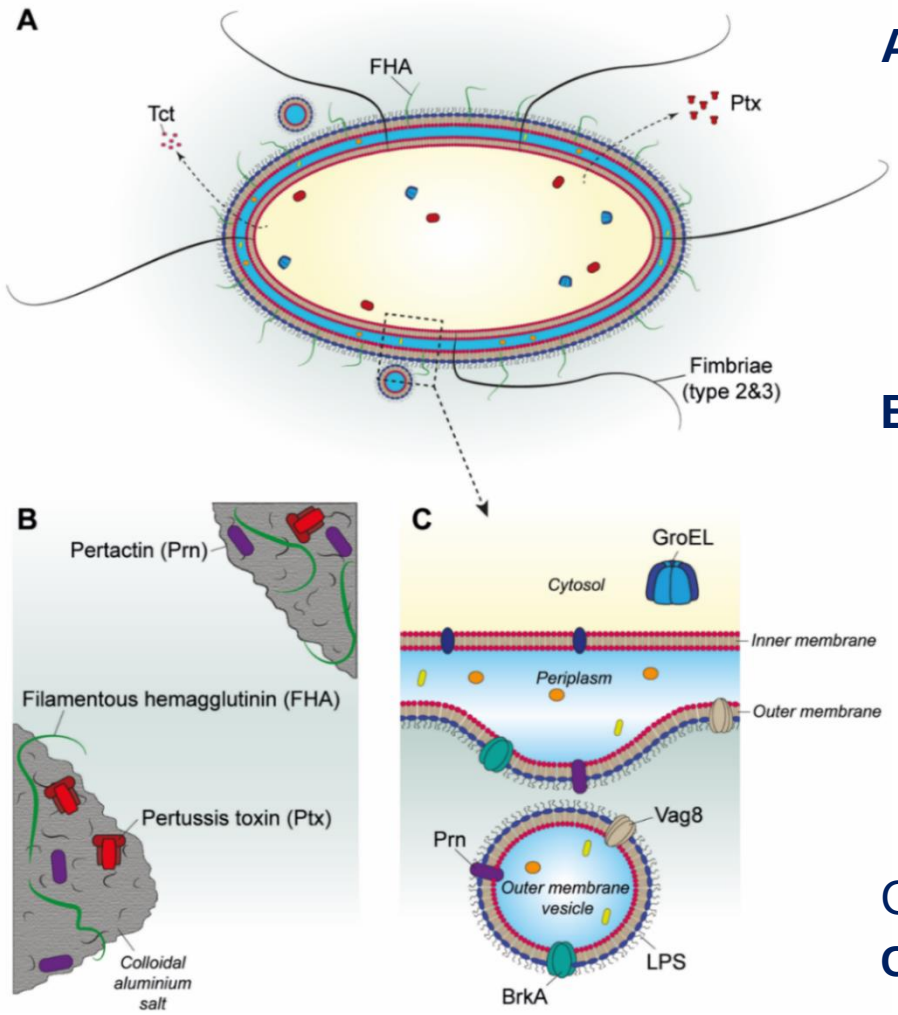


Illustration PhD thesis René Raeven

Marketed vaccines:

A. Whole cell Pertussis vaccine (wPV)

- Effective
- Cheap
- Used in 75% of the world, developing countries
- Reactogenic

B. Acellular Pertussis vaccine (aPV)

- Protects against disease
- Safe
- Expensive
- Short-term protection (Th2-biased)
- Potential lack of protection against transmission

Concept vaccine:

C. Outer membrane vesicles (omvPV)

Outer membrane vesicles (OMV)

- Ubiquitous in Gram-negative bacteria
- multiple PAMPs – provide complete immunity
- Safety:
  - cannot grow/replicate
  - Wild type strains potentially reactogenic but modifications possible
- Used as vaccine or vaccine adjuvant
- i.e pertussis OMVs provide excellent protection in mice (Raeven et al 2016) (Raeven et al 2018) (Raeven et al 2020)

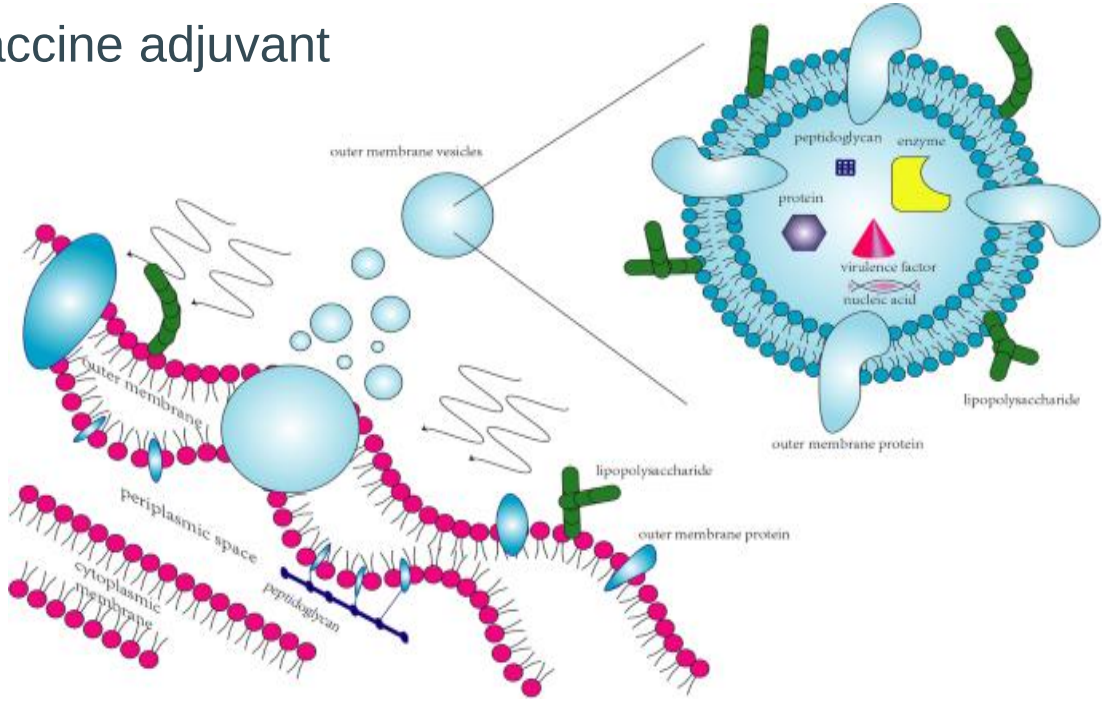


Figure from Cai W et al 2018 Diagn Pathol
https://doi.org/10.1186/s13000-018-0768-y

Pyrogen tests and omv vaccines

test	disadvantage
RPT	Procedure adjusted to be able to test samples with instrinsic pyrogenic activity
BET	Only detects endotoxin, however omv vaccines will also contain NEPs , e.g. lipoprotein

- MAT can overcome these problems
- MAT is accepted as safety and consistency test for Bexsero (vaccine containing OMV from *Neisseria Meningitidis* serogroup B) at release (Valentini et al 2019)

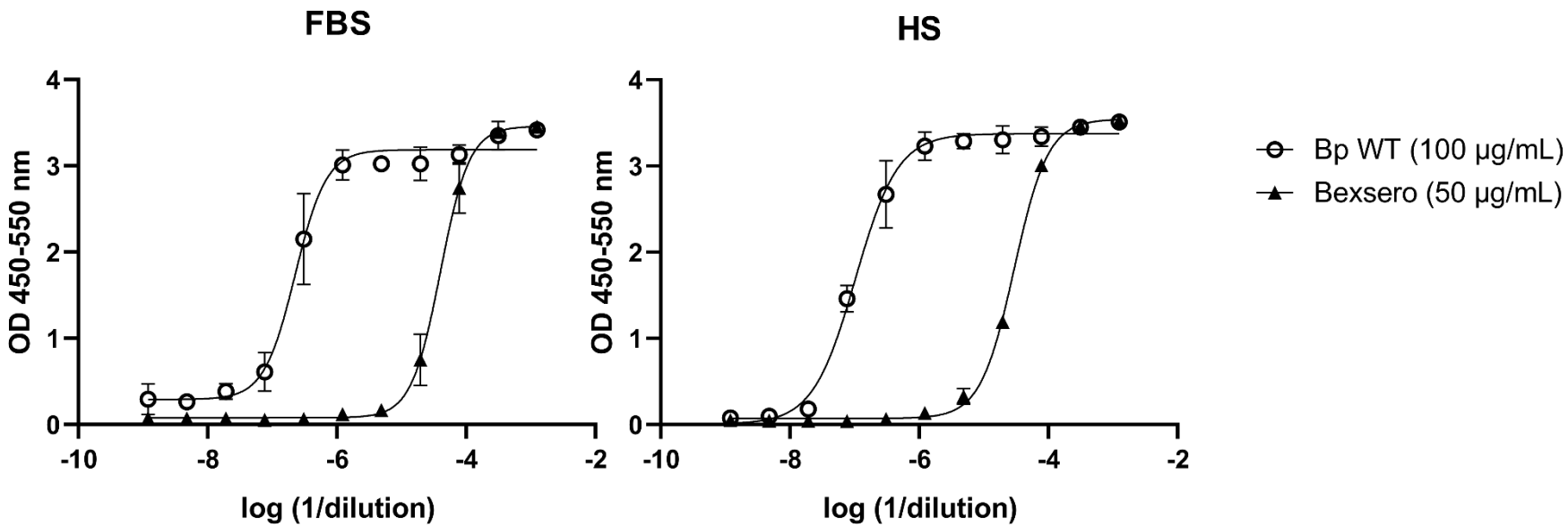
Aim and method

- Develop MAT procedure to assess reactogenicity of OMV-based preparations.
  - OMVs containing wild type (WT) *Bordetella pertussis* LPS used as model.
  - Balance between too much and too little reactogenicity

Method:

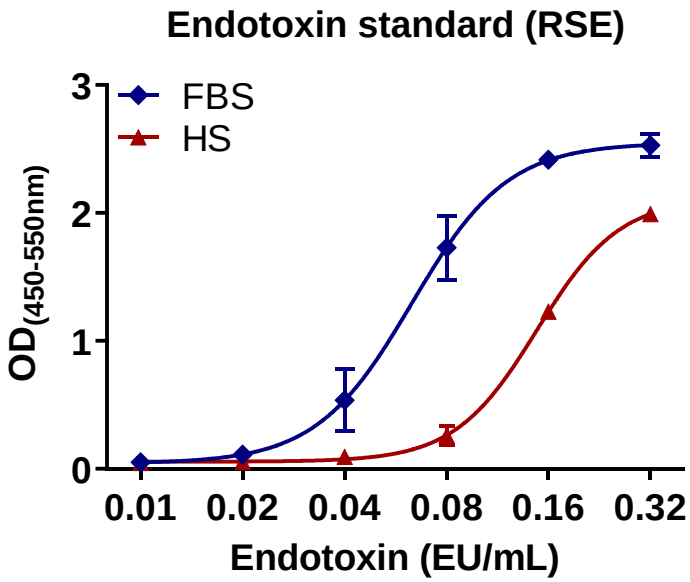
- MAT method C (Reference lot comparison)
- Bexsero as reference (proven safety profile)
- Cryopreserved pooled PBMC
- Used FBS and HS as serum source
- IL-6 ELISA read-out

Determine dilution range



- Results for Bexsero (50 µg/mL) are comparable to the reported results (Valentini et al 2019)
- Bp WT (100 µg/mL) needs to be diluted 200x more than Bexsero
- HS based MAT more sensitive than FBS for Bexsero and Bp WT

FBS vs HS as serum source in the MAT: Endotoxin



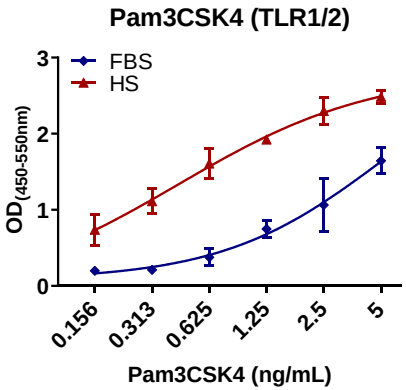
Serum source	LOD (EU/mL)
FBS	≤ 0.02
HS	≤ 0.06

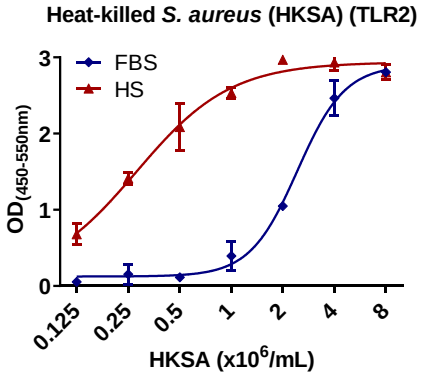
→ Lower reactivity to endotoxin with Human Serum (HS) than with Fetal Bovine Serum (FBS)

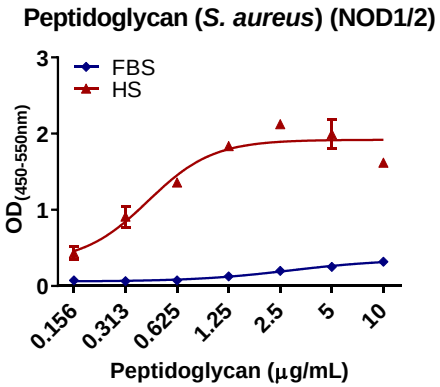
Molenaar-de Backer M.W.A. et al Altex 2020
https://doi.org/10.14573/altex.2008261

FBS vs HS as serum source in the MAT: NEPs

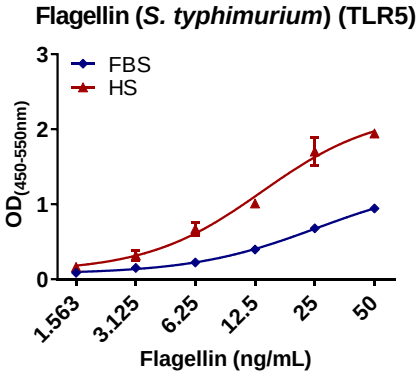
Higher reactivity with HS

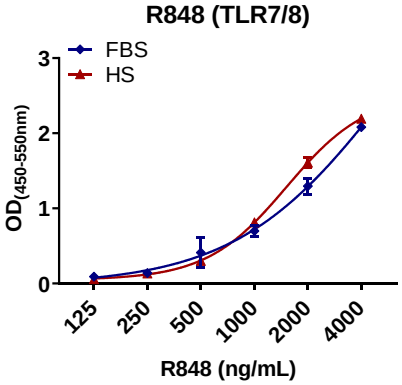






Comparable reactivity





Molenaar-de Backer M.W.A. et al Altex 2020
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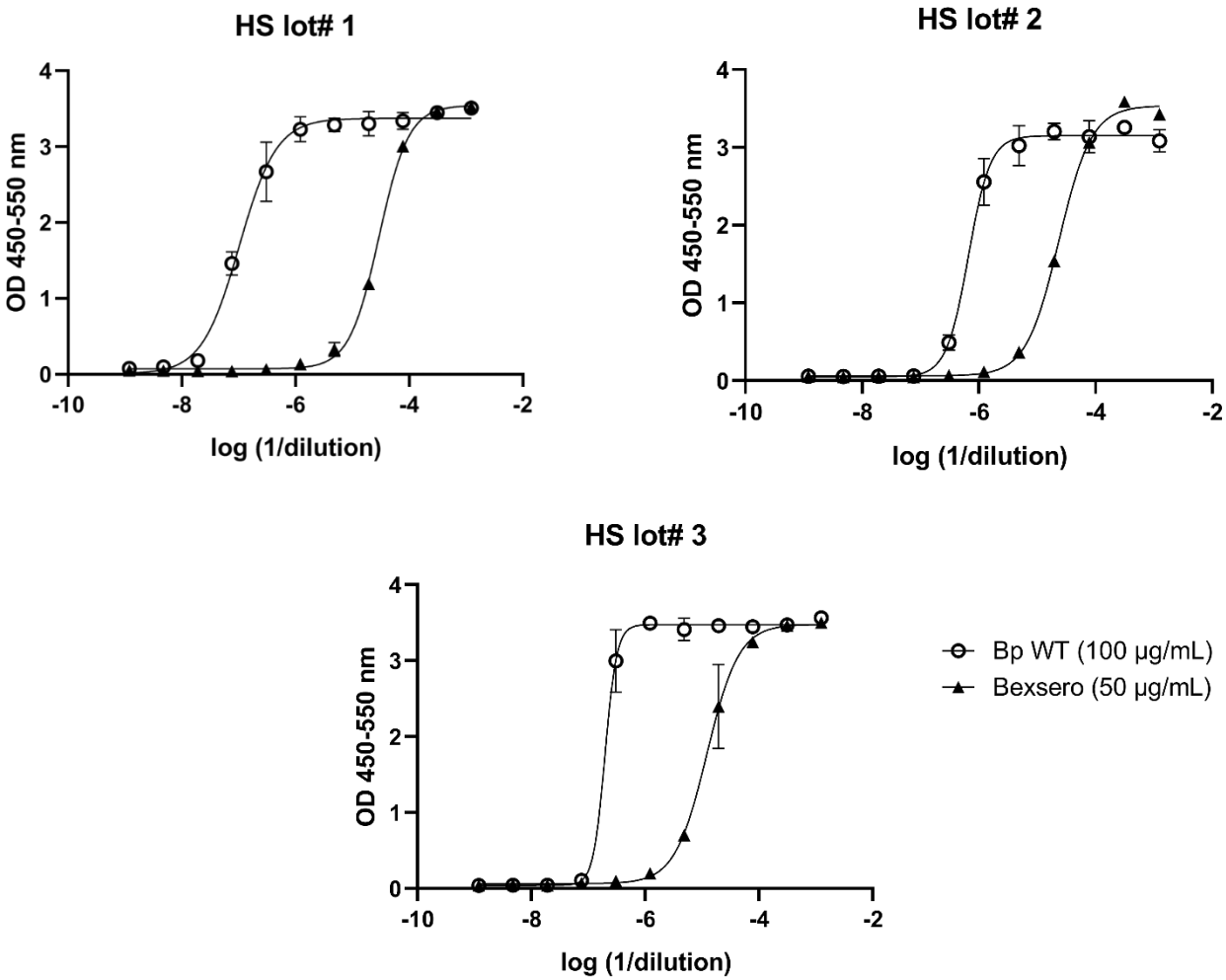
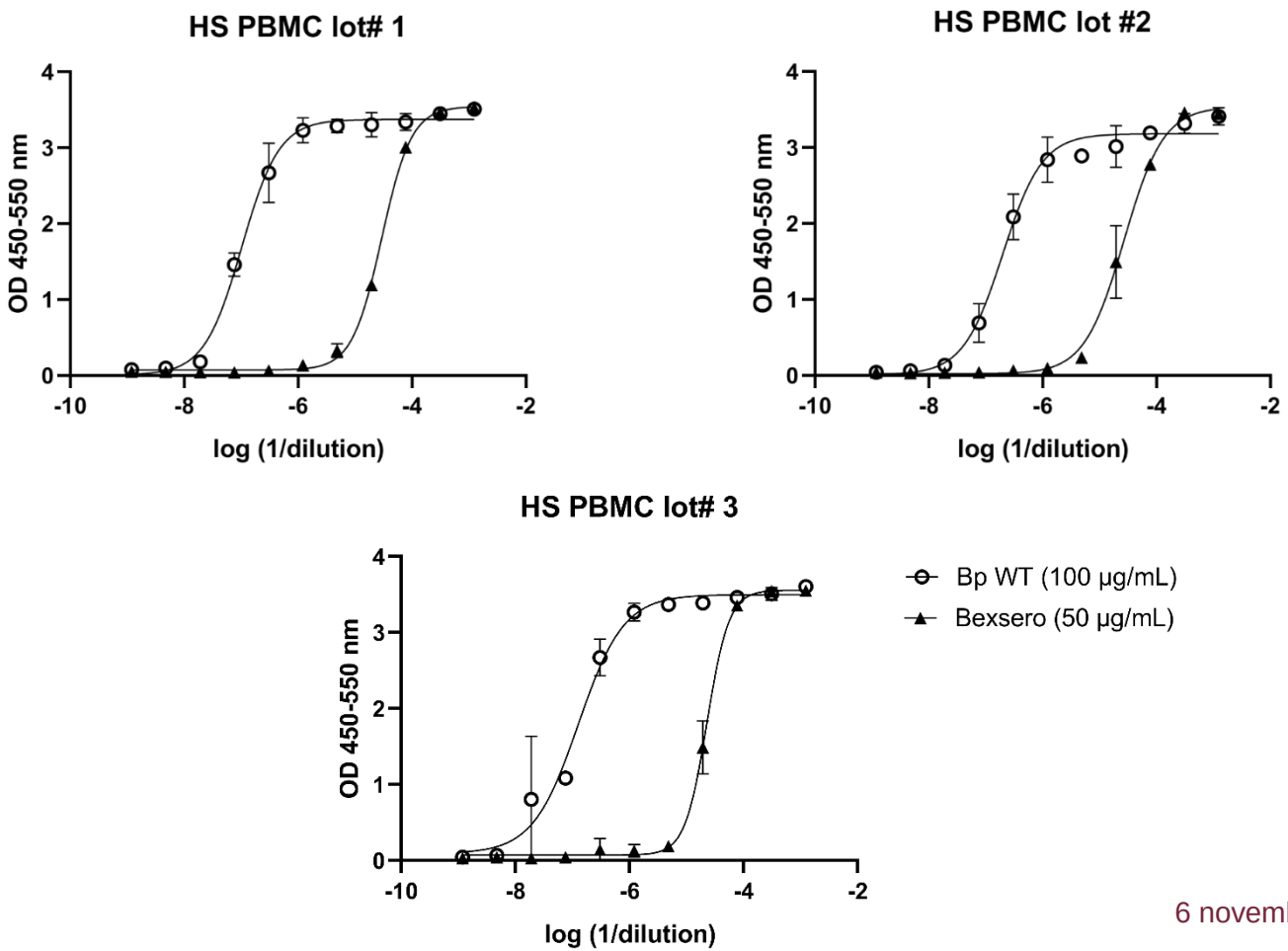
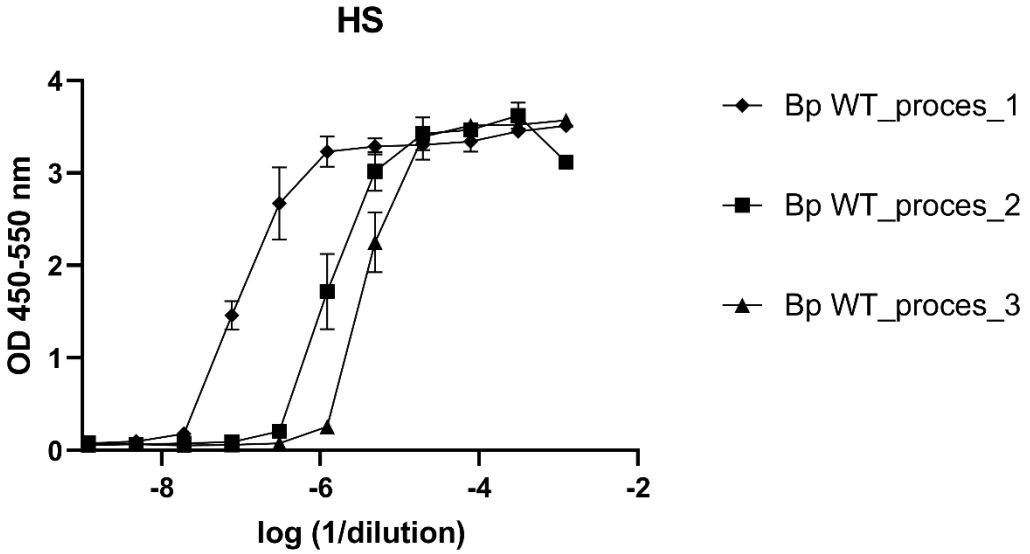
Robustness: different lots HS



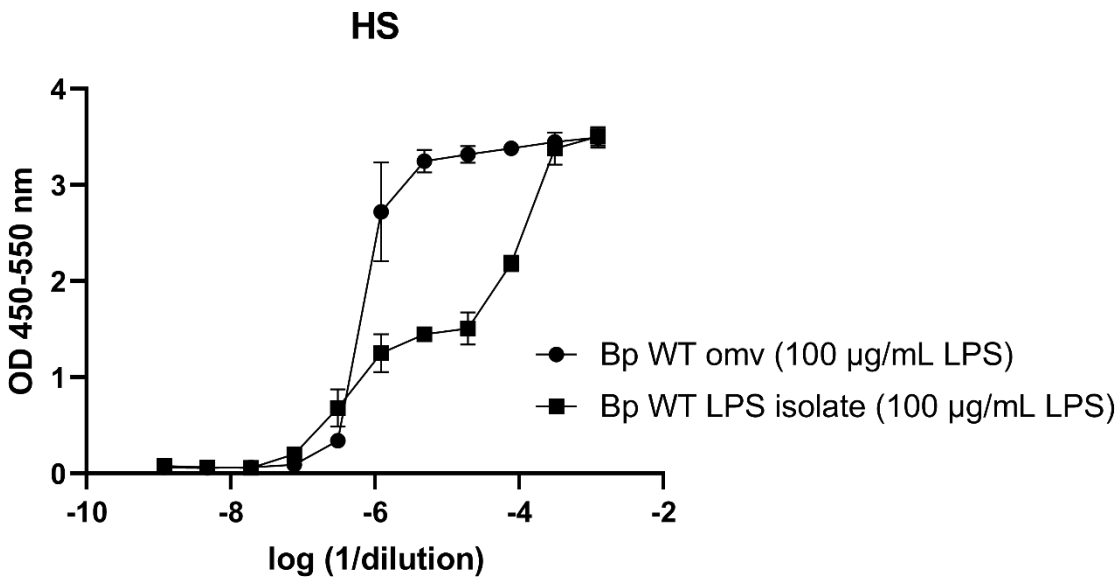
Robustness: different lots PBMCs



MAT detects production proces differences

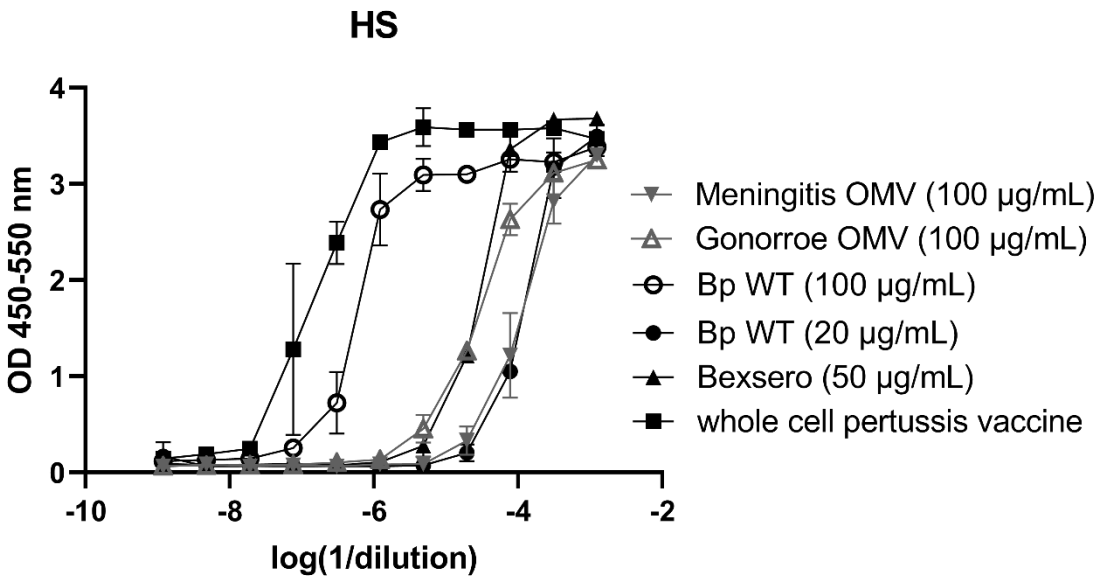


LPS isolated from *B. pertussis* vs whole OMV



→ NEPs play role in omvPV

OMV from different bacterial species



- Assay works with different products
- Assay has a broad range
- Assay able to test different concentrations and modifications

Conclusions

- MAT for OMV should be based on HS instead of FBS, since HS more sensitive in detection of NEPs.
- MAT assay is robust and can be used to determine reactogenicity in different OMV-based preparations.

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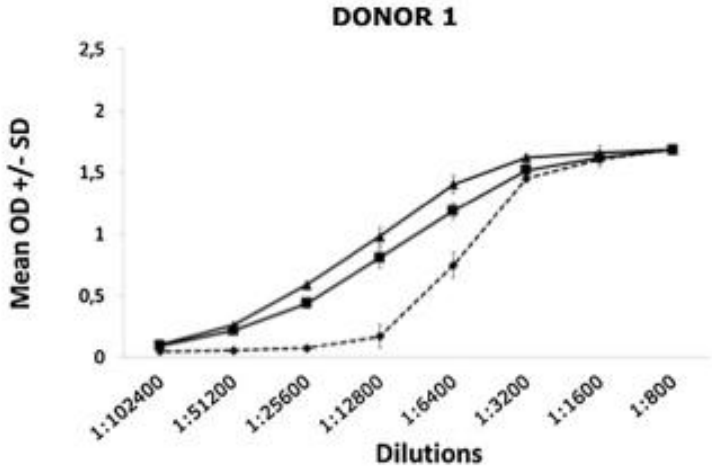
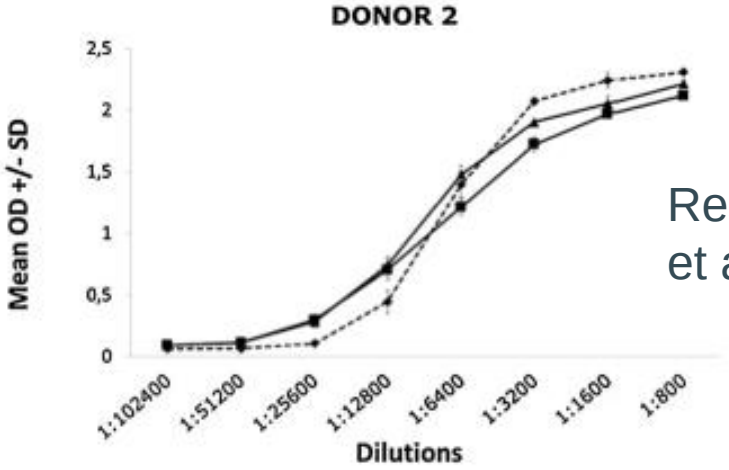
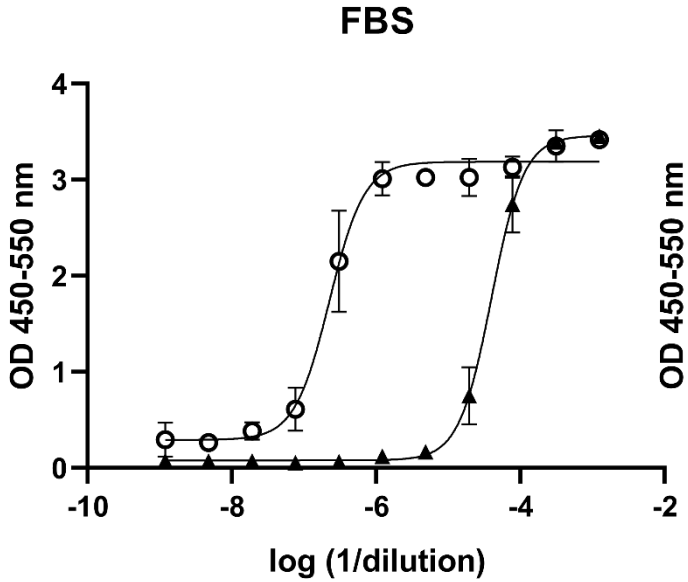
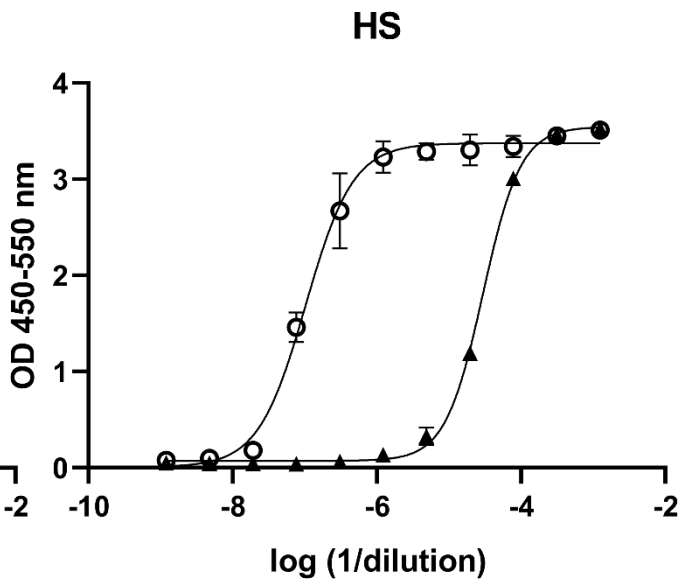
Valentini used 2-fold dilutions we used 4-fold dilutions to be in measuring range for both samples
Log(1/3200)=- 3.5
Log(1/102400) = -5.0





Results Valentin
et al 2019





Bp WT (100 µg/mL)
Bexsero (50 µg/mL)