Assessing reactogenicity of OMV-based vaccines with MAT

Marijke W.A. Molenaar-de Backer, PhD.

Senior Scientist MAT Services

m.molenaar@sanquin.nl

collaboration with:

Intravacc. Innovating vaccines

OMV-based vaccines

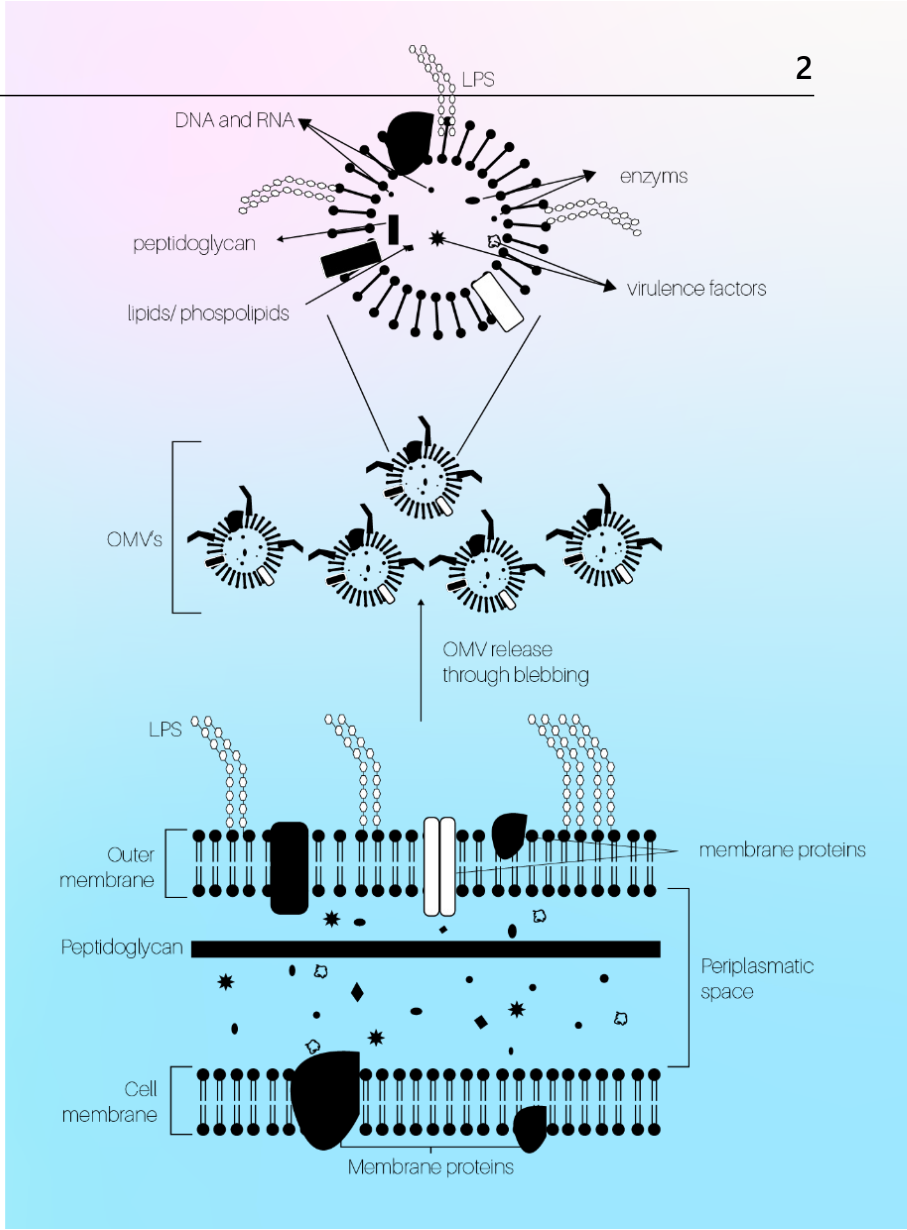
- Outer Membrane Vesicle (OMV) produced by Gram-negative bacteria
- Multiple PAMPs – provides complete immunity

- Safe – cannot grow/replicate

- Can be used as:
  - Vaccine against the bacteria itself
  - Adjuvant/delivery vehicle

- Developed as a vaccination platform by:

Intravacc.
Innovating vaccines



How to measure wanted reactogenicity when employing OMVs as vaccine?

Pyrogen tests and OMV vaccines

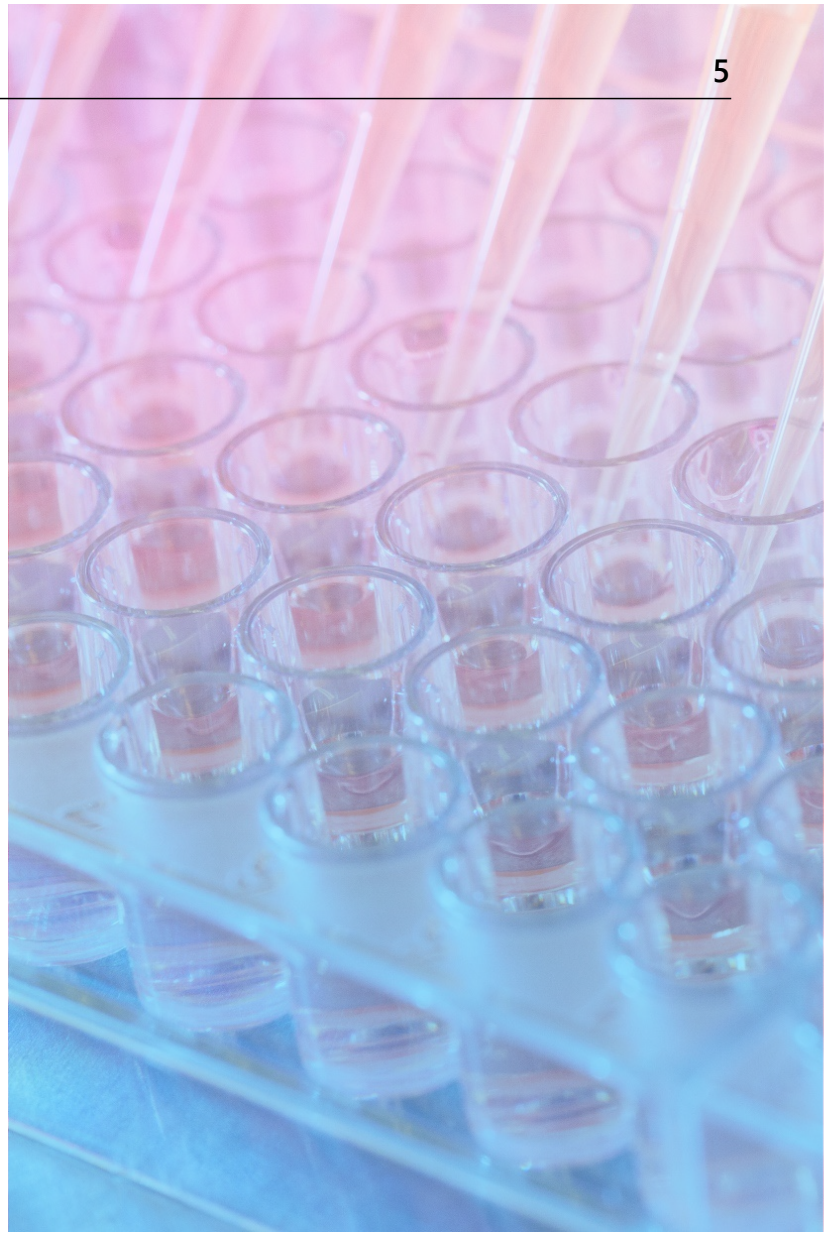
test	disadvantage
RPT	Procedure adjusted to be able to test samples with instrinsic pyrogenic activity
BET	Only detects endotoxin, however omv vaccines will also contain NEPs , e.g. lipoprotein

- 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[1]

1. **Valentini S** et al. Vaccine 2019;37(29):3754-3760. doi: **10.1016/j.vaccine.2018.10.082**

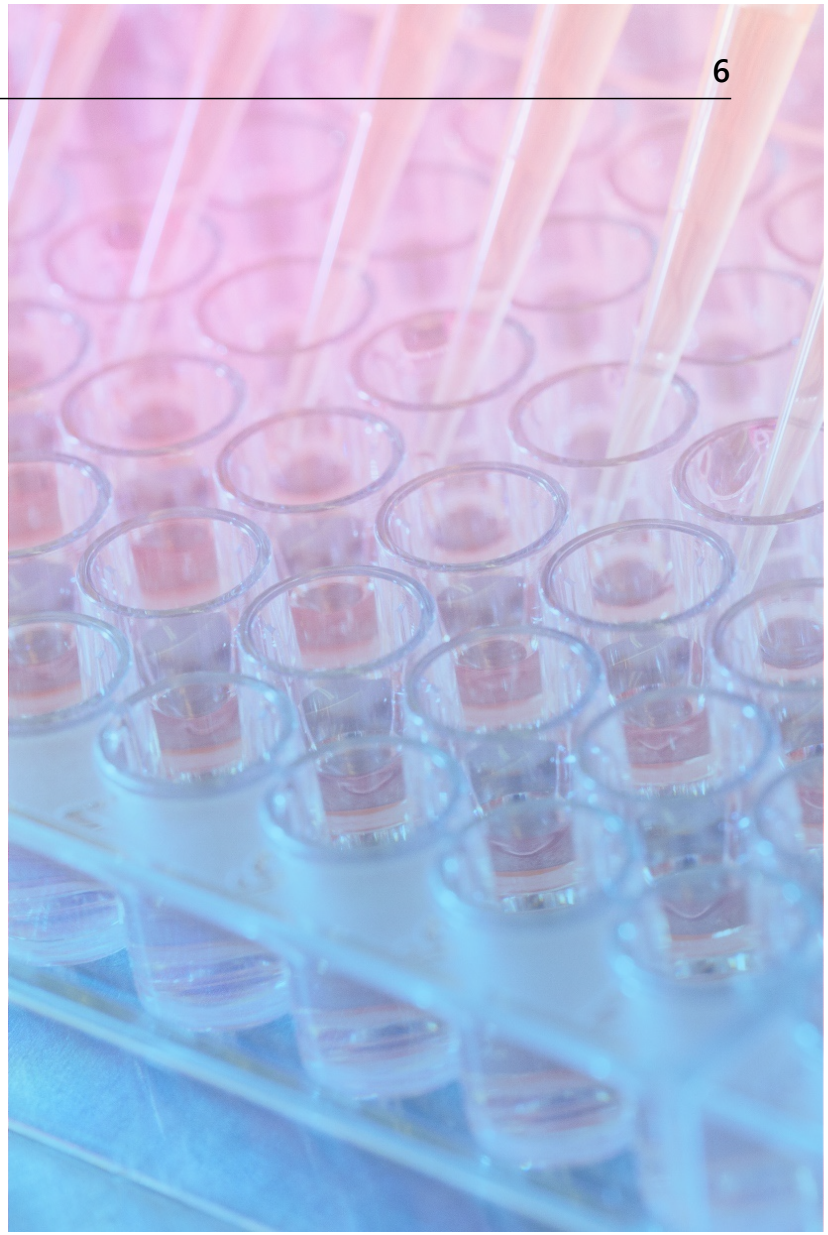
Aim

Develop MAT procedure to assess reactogenicity of OMV-based vaccine preparations



Method

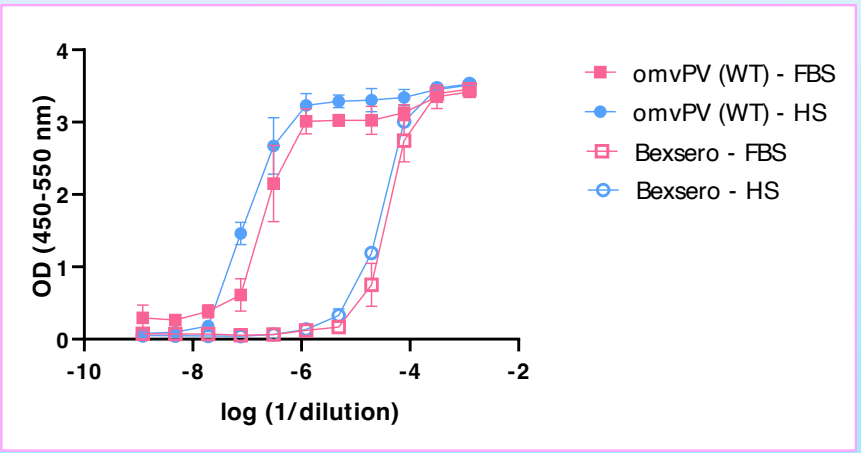
- OMVs from wild type (WT) *Bordetella pertussis*
- MAT method 2 (Reference lot comparison, previously known as method C in Ph. Eur. chapter 2.6.30/2017)
  - Problem: product in development - no reference available
  - Solution: Bexsero as reference (proven safety profile)
- Cryopreserved pooled PBMCs
- FBS and HS as serum source[1]
- IL-6 ELISA read-out



Molenaar-de Backer MWA et al. **Scientific Reports** 2023;13:12675. doi: **10.1038/s41598-023-39908-7**
1. Molenaar-de Backer MWA et al. **ALTEX** 2021;38(2):307-315. doi: **10.14573/altex.2008261**

Determining dilution range for omvPV and Bexsero

- Results for Bexsero are comparable to reported results[1]
- omvPV(WT) must be diluted 200x more than Bexsero to be in linear range of the assay
- HS-based MAT more sensitive than FBS-based MAT



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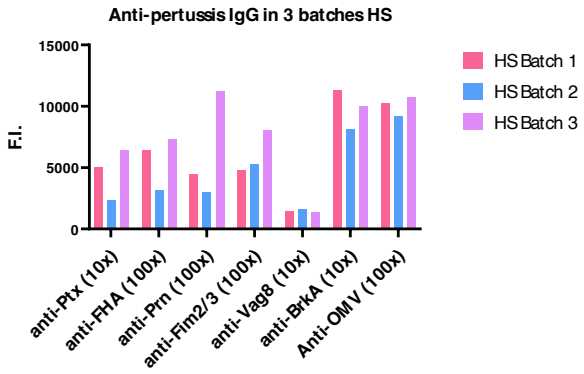
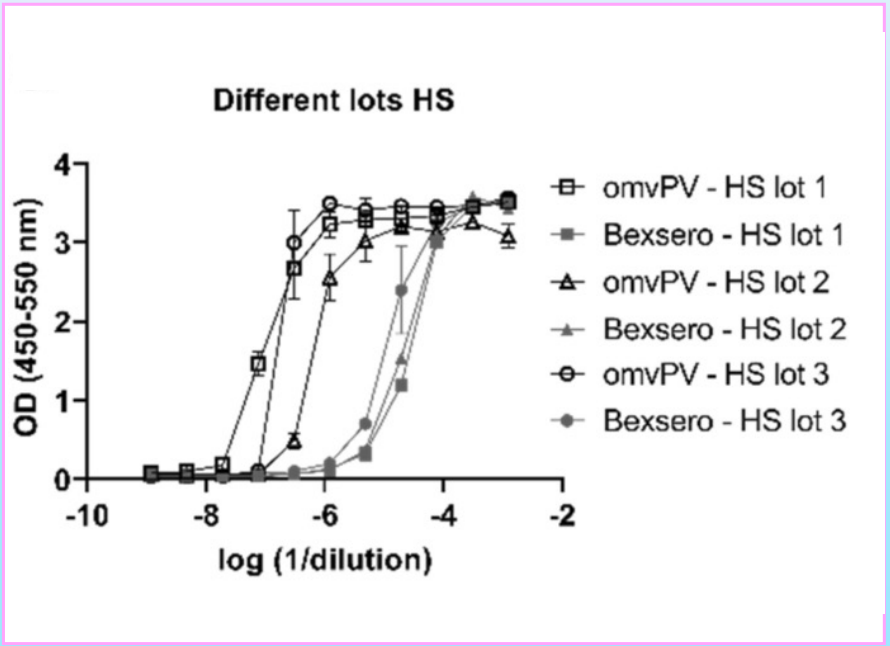
Testing robustness of the optimized assay (1)

- HS lot 2 resulted in significantly lower reactogenicity for omvPV, probably due to difference in anti-pertussis antibodies. The IL-6 results for Bexsero were not affected by HS lot 2.





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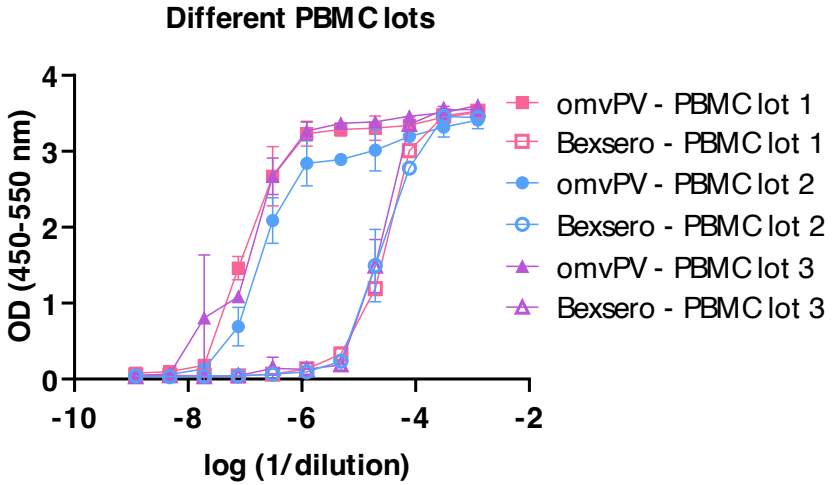
Testing robustness of the optimized assay (2)

- HS lot 2 resulted in significantly lower reactogenicity for omvPV, probably due to difference in anti-pertussis antibodies. The IL-6 results for Bexsero were not affected by HS lot 2.
- Comparable reactogenic results for different PBMC lots



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Testing robustness of the optimized assay (3)

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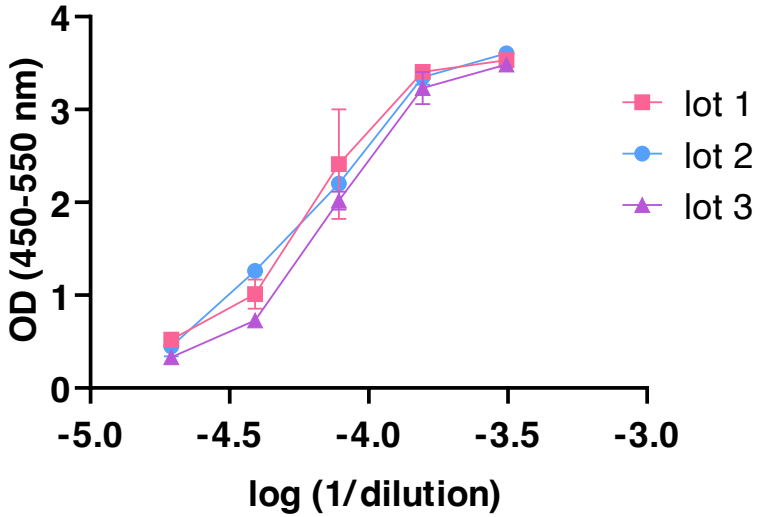
- Comparable reactogenic results for different PBMC and Bexsero lots



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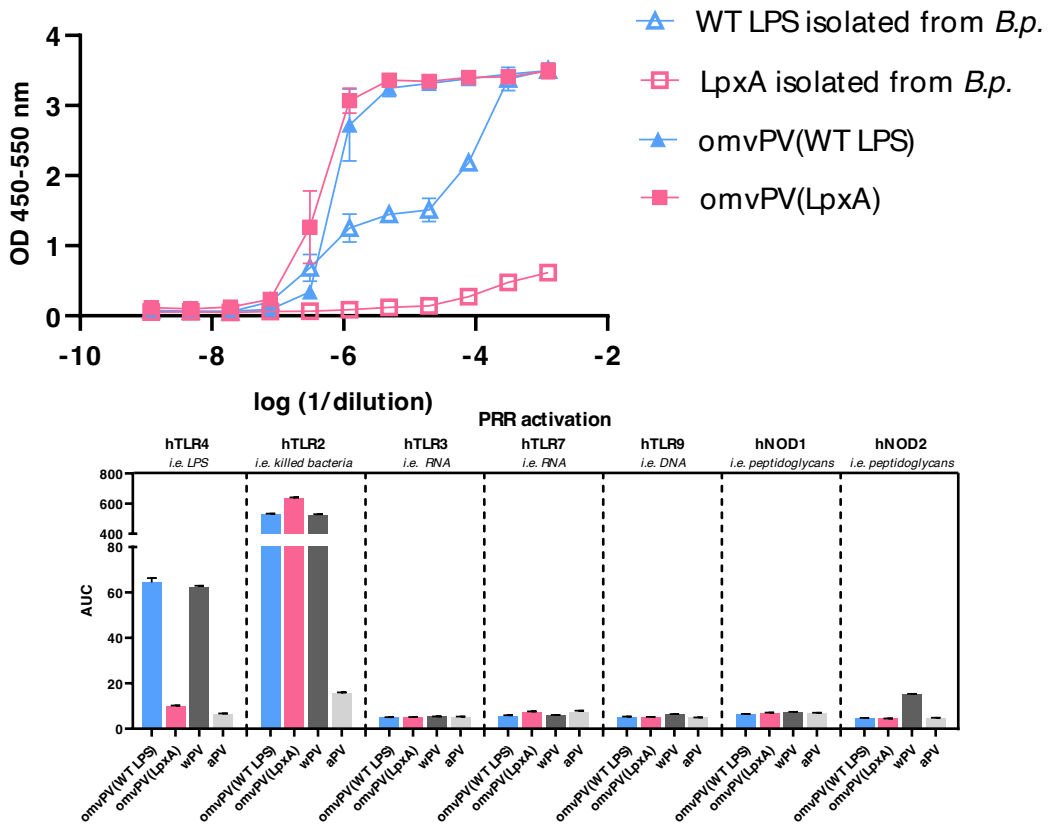
Effects of LPS modification on MAT reactogenicity

- LPS modification decreases reactogenicity (compare WT LPS with LpxA)
- When LPS modification is present in OMV then no effect on reactogenicity (compare omvPV (WT LPS) and omvPV(LpxA).
  - ➢ probably due to increased TLR2 reactogenicity



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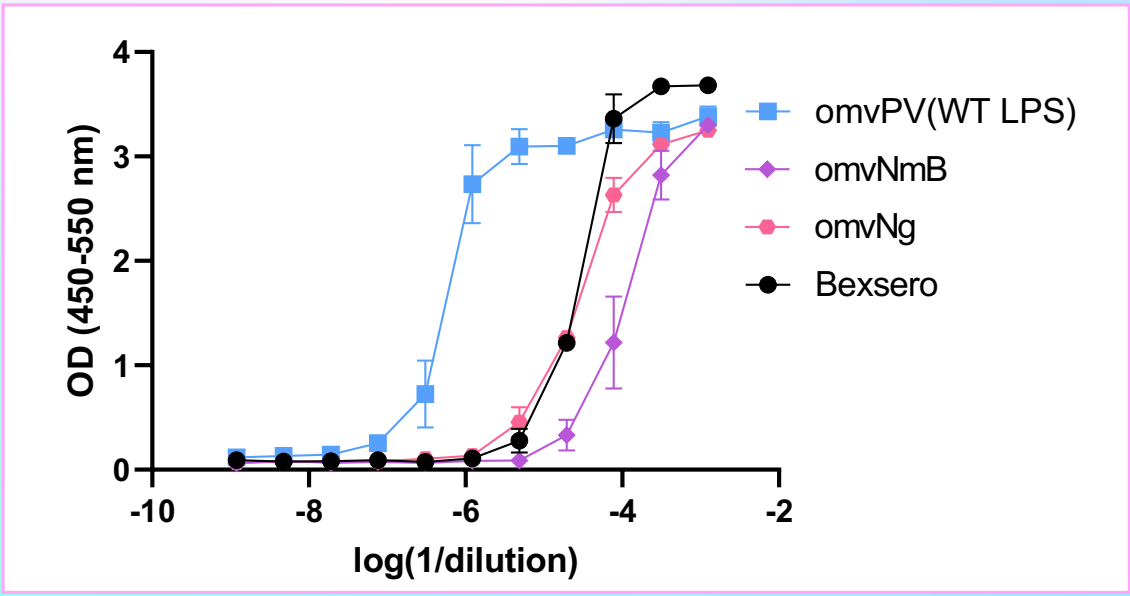
Using the MAT to test OMVs from different bacterial species

- Assay works with different bacterial species
- Assay has a broad range



NmB => Neisseria Meningitidis serogroup B
Ng => Neisseria Gonorrhoeae

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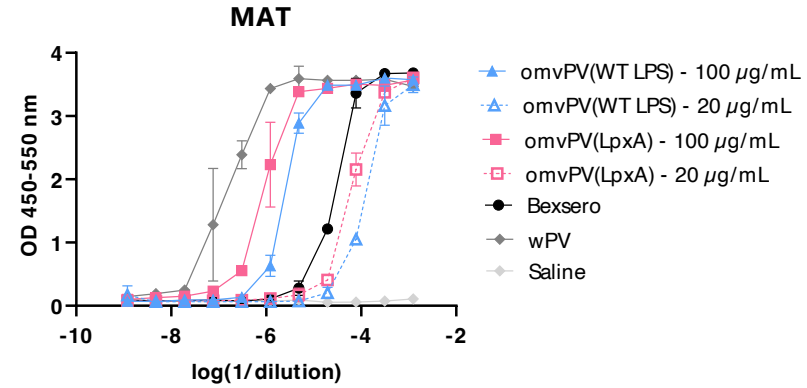
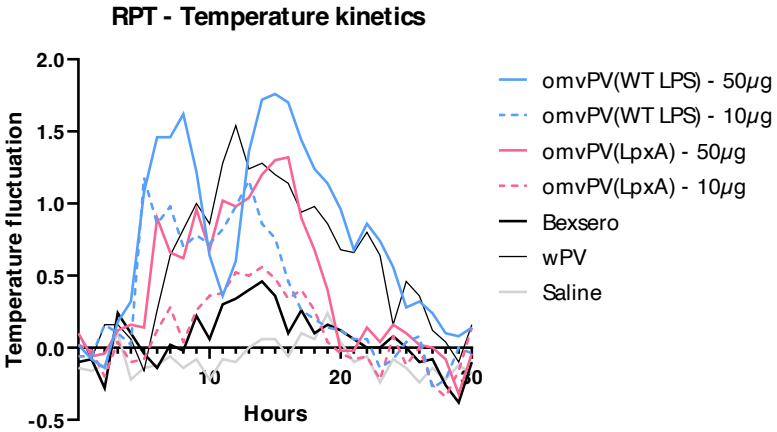
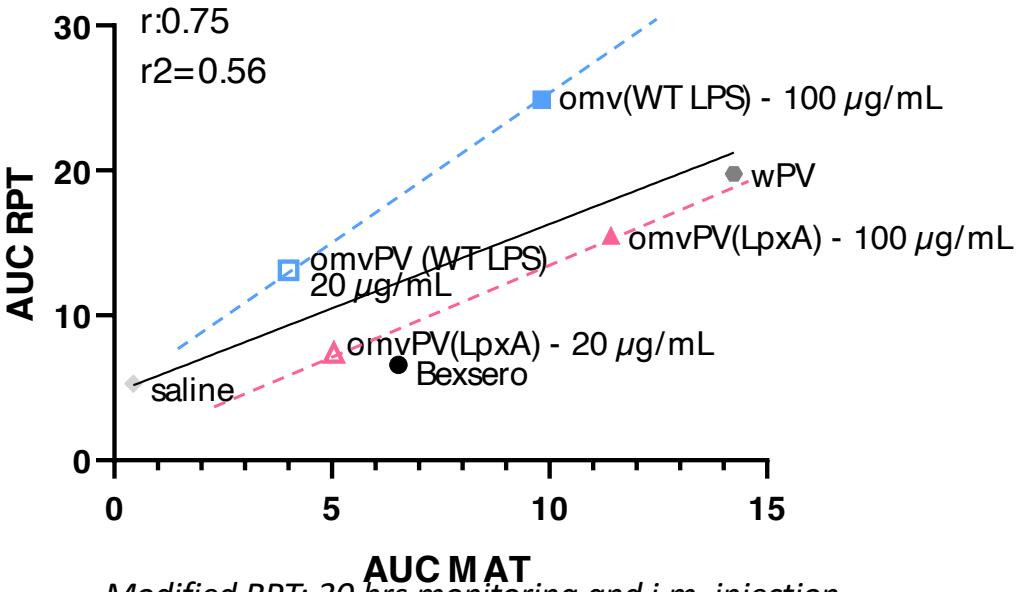
Comparing MAT and modified RPT for OMV testing







Modified RPT: 30 hrs monitoring and i.m. injection.

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Conclusions

- MAT assay is robust and can be used to determine reactogenicity during vaccine development and in different OMV-based preparations
- Alternative reference lot can be used during development
- MAT for OMV should HS-based instead of FBS, as HS allows for more sensitive detection of NEPs, but FBS can be needed if (specific) antibodies in HS interact with the product
- Good correlation beween MAT and modified RPT assay

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