Hijacking the Monocyte Activation Test (MAT)

from Pyrogen test to Supporting Immunogenicity Testing

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collaboration with: Intravacc. Innovating vaccines

Early-stage vaccine development

- Vaccine design will depend on:
  - Pathogen or antigen of interest;
  - Antibody titers;
  - T cell response;
- Does a vaccine candidate or platform activate the immune system?
- Is it generating an antigen-specific response?

Early-stage vaccine development

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  - Pathogen or antigen of interest;
  - Antibody titers;
  - T cell response;
- **Does a vaccine candidate or platform activate the immune system?**
- Is it generating an antigen-specific response?

OMV-based vaccines

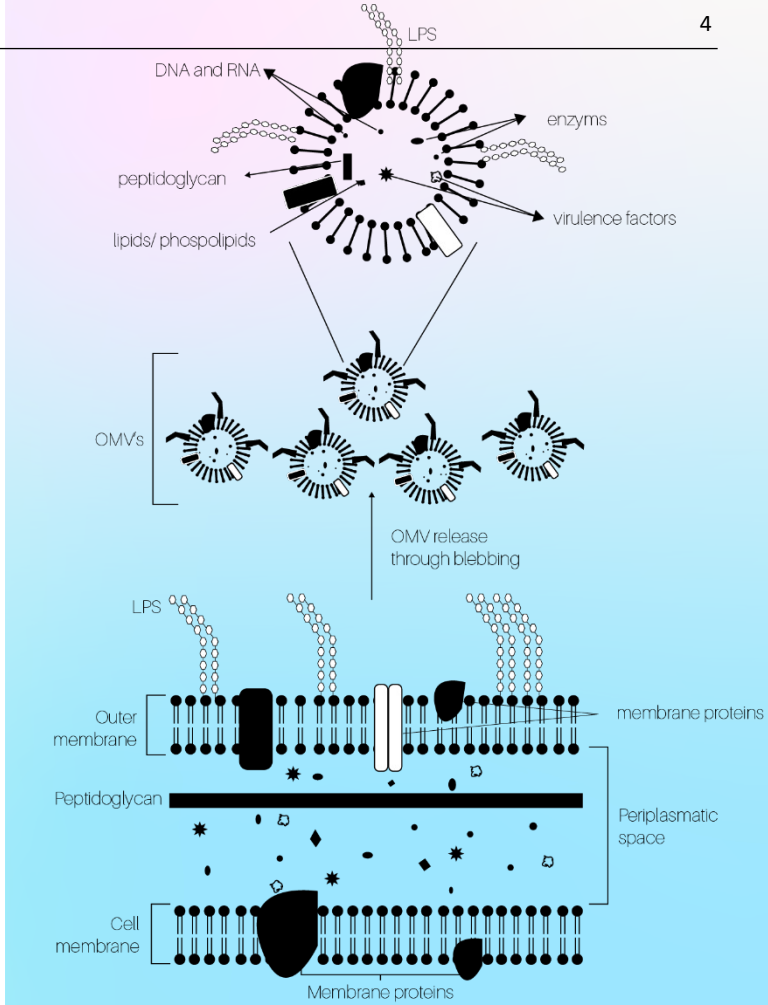
- Outer Membrane Vesicle (OMV) produced by Gram-negative bacteria
- Multiple PAMPs – provides complete immunity

- 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 adjuvants?

Pyrogen tests and OMV vaccines

Test	Disadvantage
Rabbit Pyrogen Test (RPT)	Procedure needs to be adjusted to be able to test samples with instrinsic pyrogenic activity
Bacterial Endotoxin Test (BET)	Only detects endotoxin, however OMV vaccines will also contain non-endotoxin pyrogens, e.g. lipoprotein

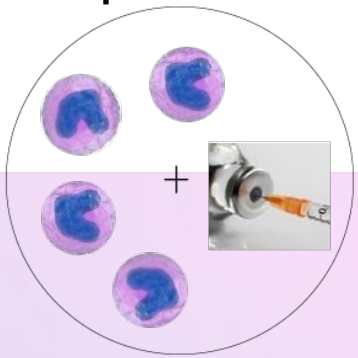
- MAT can overcome these problems
- MAT is accepted as safety and consistency test for Bexsero (vaccine containing OMV from *Neisseria Meningitidis* serogroup B) at release[1]

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

Monocyte Activation Test (MAT)

- MAT is an in-vitro pyrogenicity test for parenteral medicines and replaces the rabbit pyrogen test
- MAT can also be used to determine reactogenicity of a vaccine
- determine batch consistency at release

'Fever in a plate'



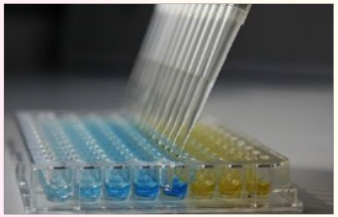
Incubation at 37° c

cytokines

IL 6, IL 1ß, TNFα

fever

ELISA



Human white blood cells (monocytes)

Uses cryopreserved pooled PBMCs and IL-6 as readout

Aim

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

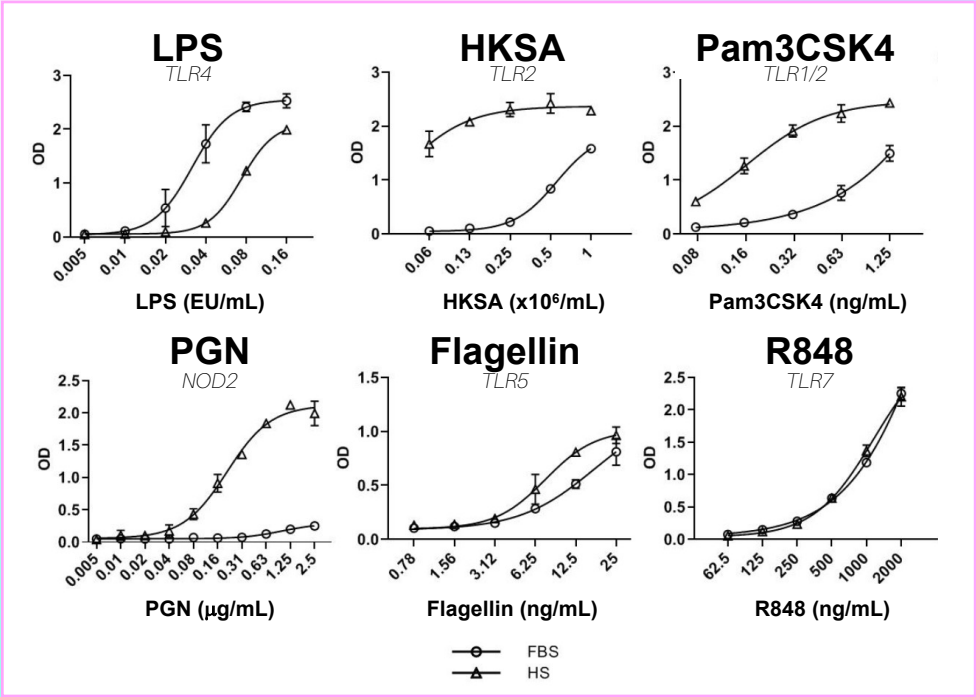
Method

- OMVs containing wild type (WT) *Bordetella pertussis* LPS
- MAT method C (Reference lot comparison)
  - Problem: product in development - no reference available
  - Solution: Bexsero as reference (proven safety profile)
- Cryopreserved pooled PBMC
- 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

Assessing impact of different serum sources in MAT

- Lower reactivity towards LPS with Human Serum (HS) compared with Fetal Bovine Serum (FBS)
- Higher reactivity towards HKSA, Pam3CSK4 and PGN with HS than with FBS
- Comparable reactivity for FLA-ST and R848



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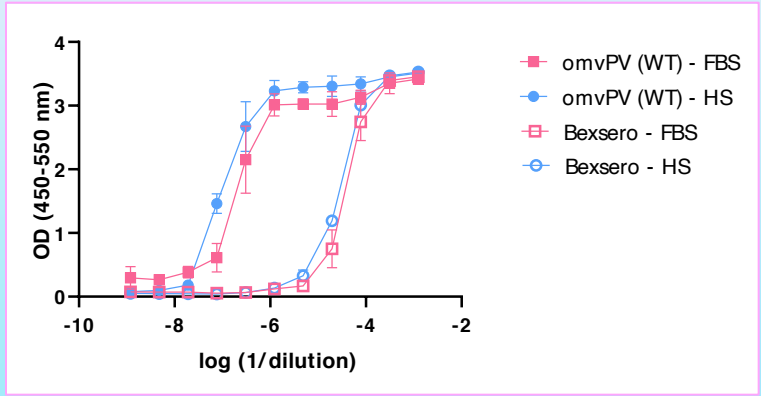
Determining dilution range for omvPV and Bexsero

- 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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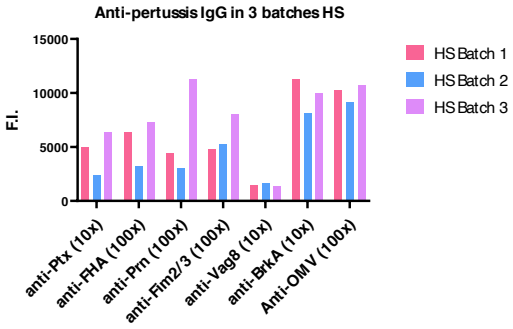
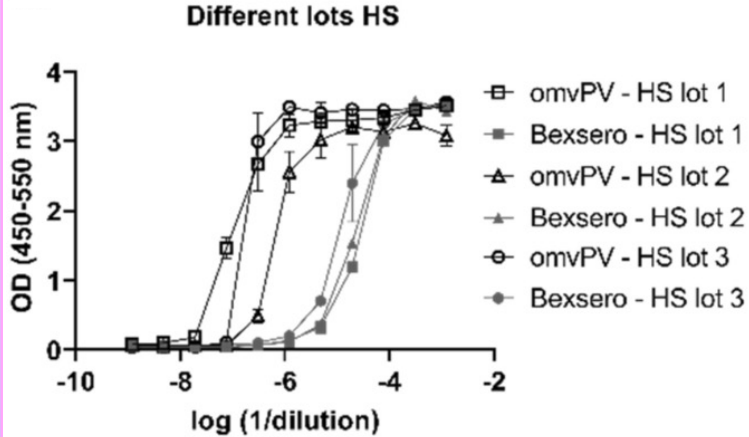
Testing robustness of the optimized assay (1)

- 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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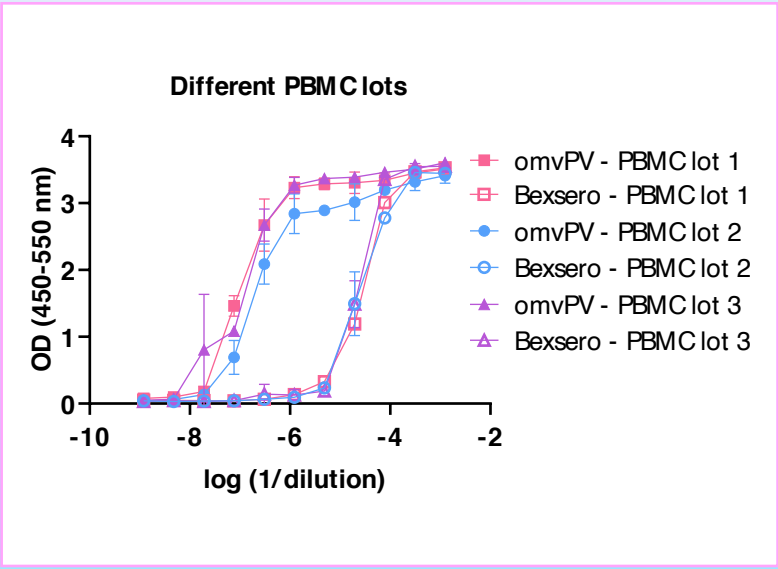
Testing robustness of the optimized assay (2)

- 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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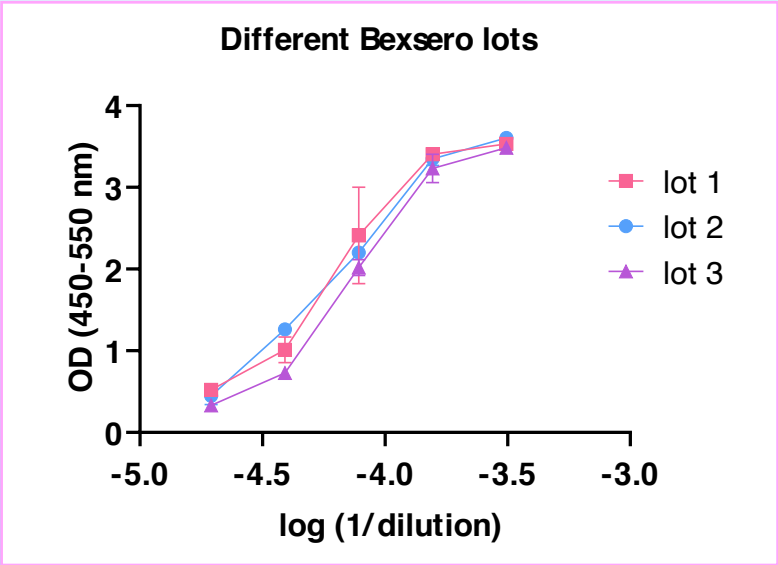
- Comparable reactogenic results for different PBMC and Bexsero lots



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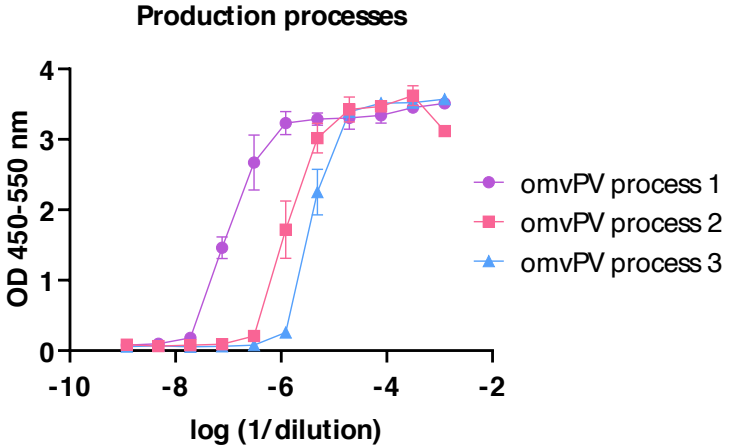
Testing robustness of the optimized assay (4)

- 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 and Bexsero lots
- MAT can also be used to screen for changes in production process



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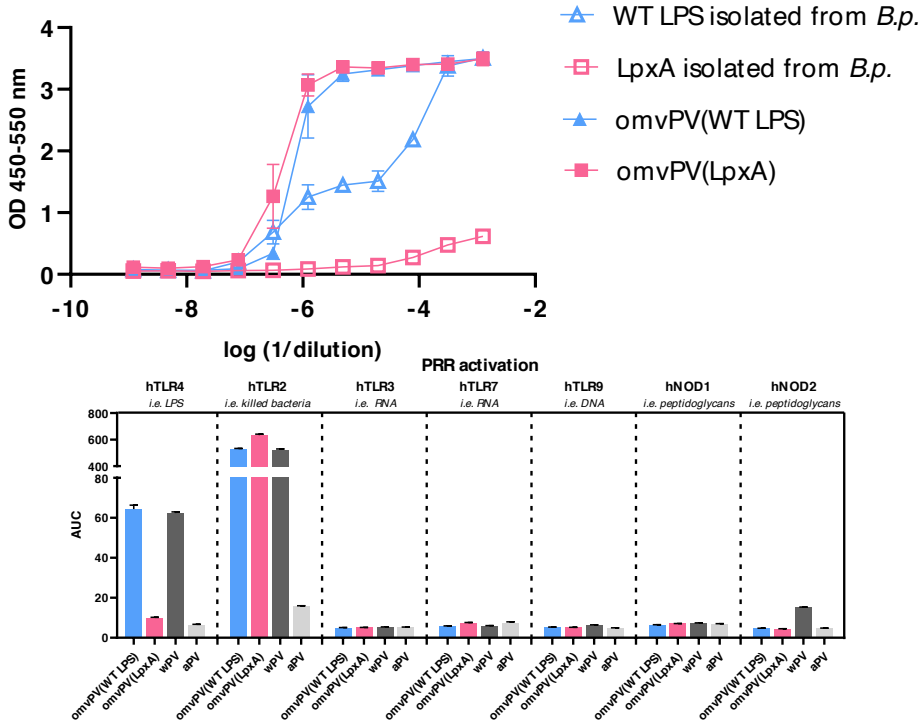
Effects of LPS modification on MAT reactogenicity

- 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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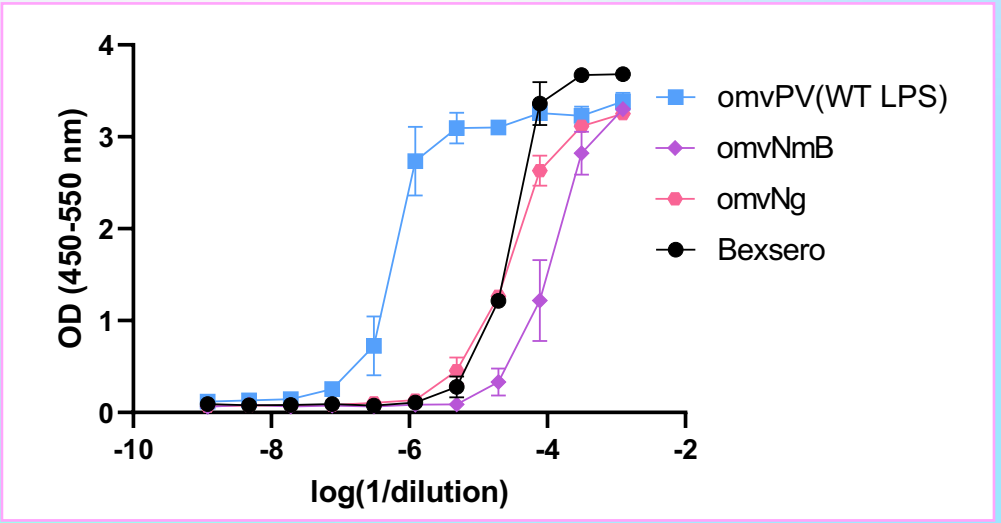
Using the MAT to test OMVs from different bacterial species

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



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

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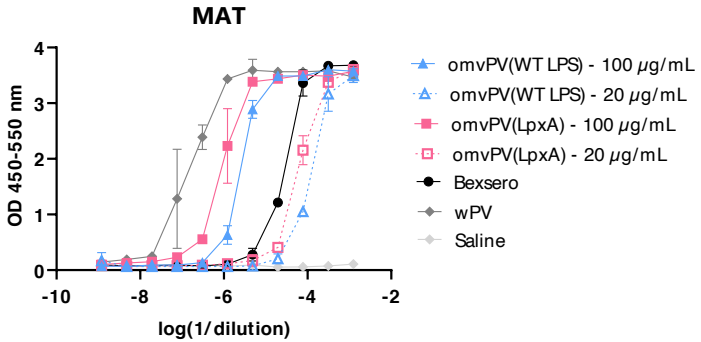
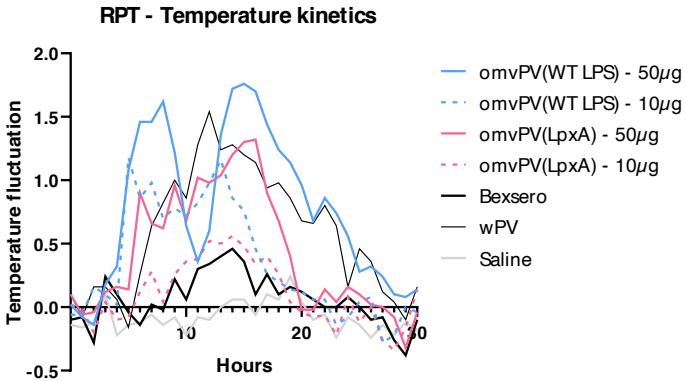
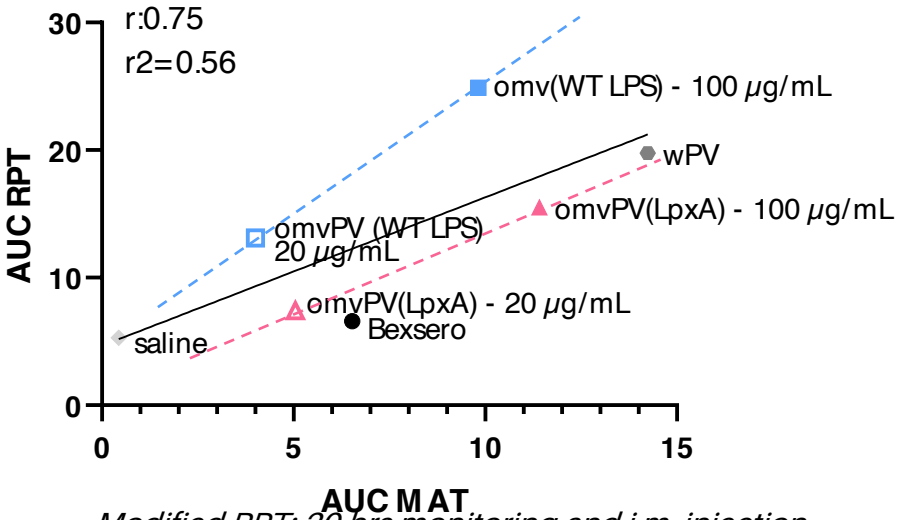
Comparing MAT and modified RPT for OMV testing







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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Intravacc

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