Norwegian Medical Products Agency

Implementing MAT for a Pyrogenic Vaccine

September 25th, 2024

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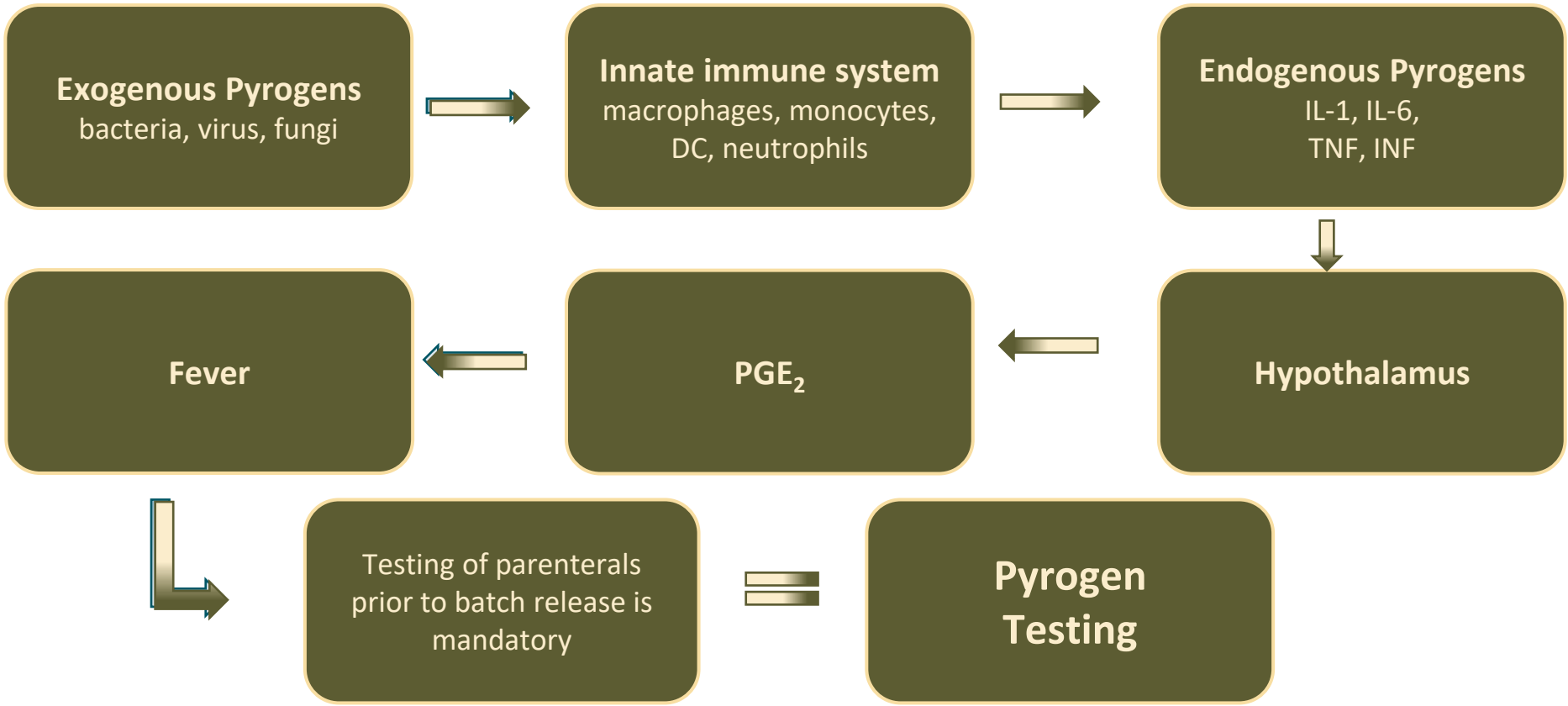
Outline

- What are pyrogens?
- Bexsero - 4CMenB Vaccine
- RPT & LAL
- Ph. Eur. 2.6.30. Monocyte-activation test, Method 2
- MAT testing at NoMA
- Donor to donor variability
- Product specific validation
- Specification setting for an inherently pyrogenic vaccine
- OOS Investigation
- Ph. Eur. 2.6.40. Monocyte-activation test for vaccines containing inherently pyrogenic components

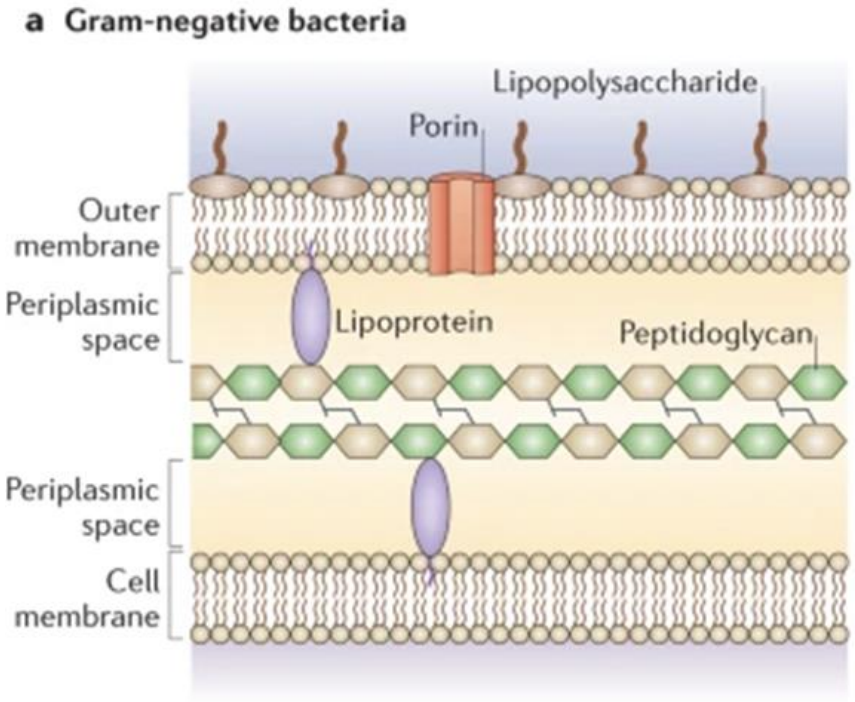
What are pyrogens?

A substance that produces a rise in temperature in a human or an animal



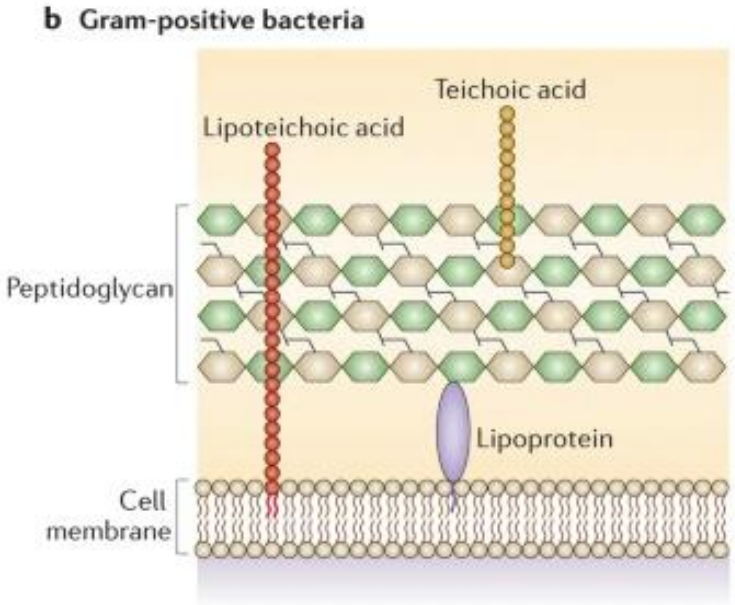
Endotoxin Pyrogens

- LPS known as endotoxin
- Component of outer cell membrane of gram-negative bacteria



Non-endotoxin Pyrogens (NEP)

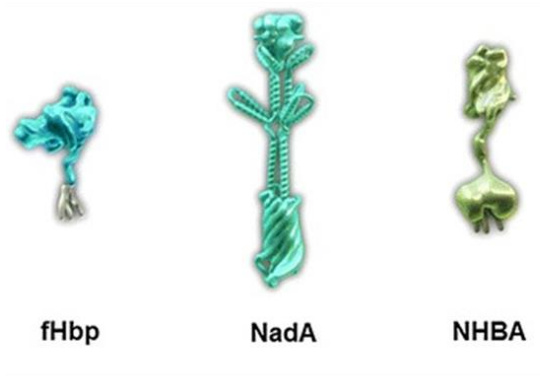
- peptidoglycan, lipoteichoic acids and bacterial lipoproteins
- Viruses
- Yeast & funghi
- Non-biological sources

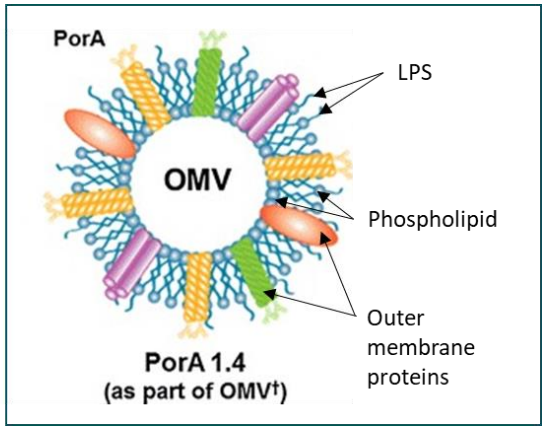


Brown, L., Wolf, J., Prados-Rosales, R. et al. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. Nat Rev Microbiol 13, 620–630 (2015).

Bexsero - 4CMenB Vaccine

- The first multi-component, protein-based vaccine against Neisseria meningitidis serogroup B
- Contains three recombinant proteins and OMV
- fHbp – factorH binding protein
- NadA – Neisserial adhesin A
- NHBA – Neisseria heparin-binding antigen
- OMV – Outer membrane vesicles, from the New Zealand outbreak strain (NZ 98/254)
- All components are adsorbed onto aluminum hydroxide





OMV are purified from the bacterial membranes and are inherently pyrogenic, containing

- endotoxin (meningococcal lipopolysaccharide)
- non-endotoxin (e.g. lipoproteins)

fever-inducing components

O'Ryan M, Stoddard J, Toneatto D, Wassil J, Dull PM. A multi-component meningococcal serogroup B vaccine (4CMenB): the clinical development program. Drugs (2014) 74:15-30.

MAT Implementation for testing of Bexsero at NoMA

2013

- MA granted by EMA for Bexsero
- Endotoxin/Pyrogenicity of final lot established by LAL and RPT
- NoMA and NIBSC – OCABR release of Bexsero (LAL)

RPT

- Ph. Eur. Pyrogens (2.6.8)
- Detects endotoxin & non-endotoxin pyrogens
- High failure rate at manufacturer and OMCL

LAL

- Ph. Eur. BET (2.6.14)
- Detects ONLY endotoxin pyrogens
- Ph. Eur. Guidelines for using the BET (5.1.10)
- High variability in results and high failure rate at OMCLs

MAT

- Ph. Eur. Monocyte Activation Test (2.6.30)
- **Detects endotoxin & non-endotoxin pyrogens**
- NoMA/NIBSC/manufacturer collaboration – implemented MAT to replace LAL/RPT

2017

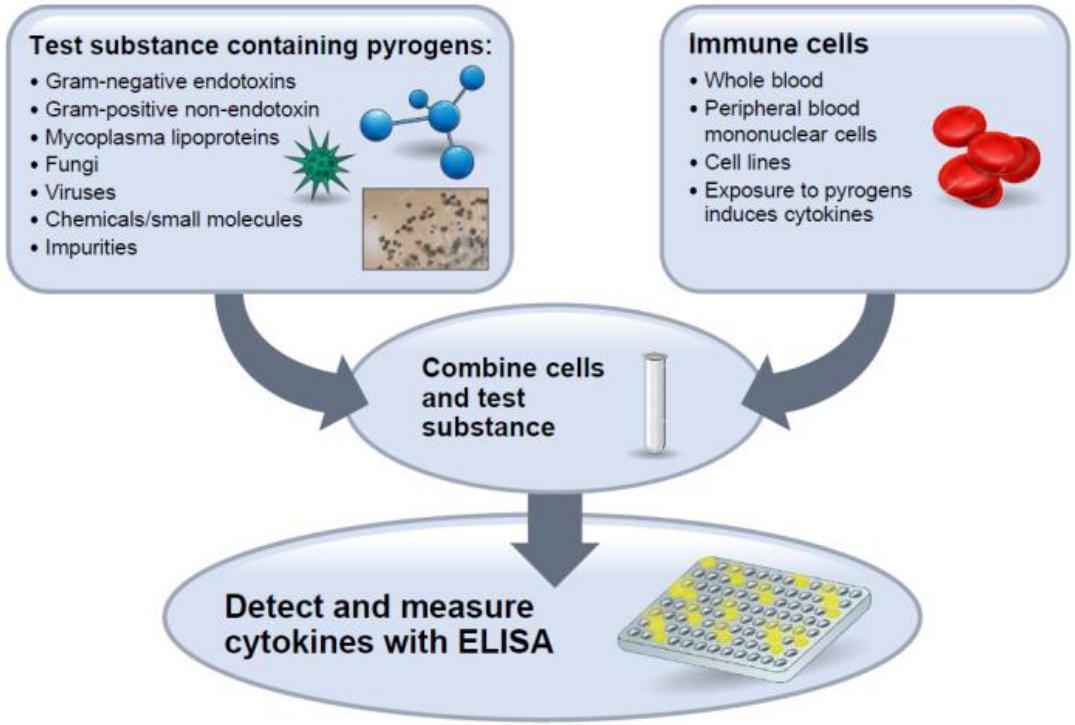
- MAT method implemented by manufacturer
- NoMA performed OCABR release using MAT

- Vaccine, 2019 Jun 27;37(29):3747-3753
- Vaccine, 2019 Jun 27;37(29):3754-3760
- Vaccine, 2019 Jun 27;37(29):3761-3769

Ph. Eur. 2.6.30 – Monocyte-activation Test - MAT

2 Methods described in chapter 2.6.30

- Method 1 (Semi-quantitative): comparison of test sample with a standard endotoxin dose-response curve
- **Method 2 (Reference lot comparison test): comparison of the test sample with a validated reference lot of that preparation**

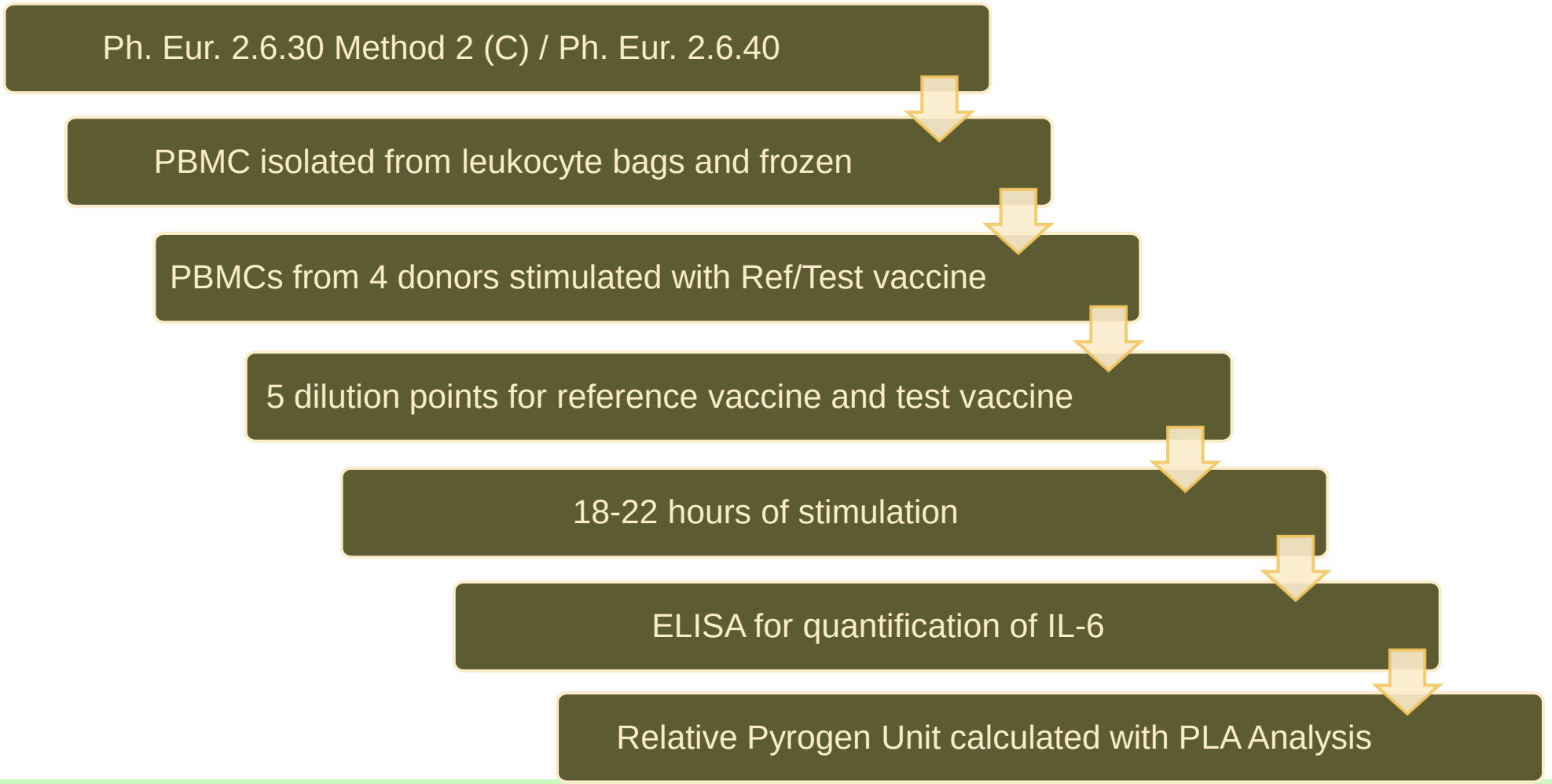


Different variants of MAT

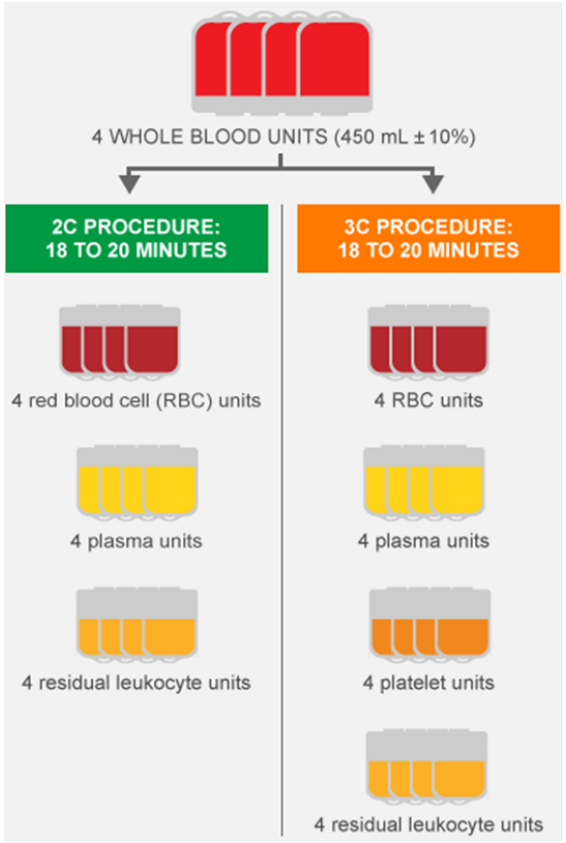
- Source of human monocytes
  - Whole blood (fresh or cryopreserved)
  - PBMCs (fresh or cryopreserved)
  - Human monocytic cell line (Mono-Mac 6)
- ELISA read-out
  - IL-6, IL-1β, TNF-α

Allen et al. —MAT workshop March 2019, NICEATM poster: SOT 2019 Annual Meeting

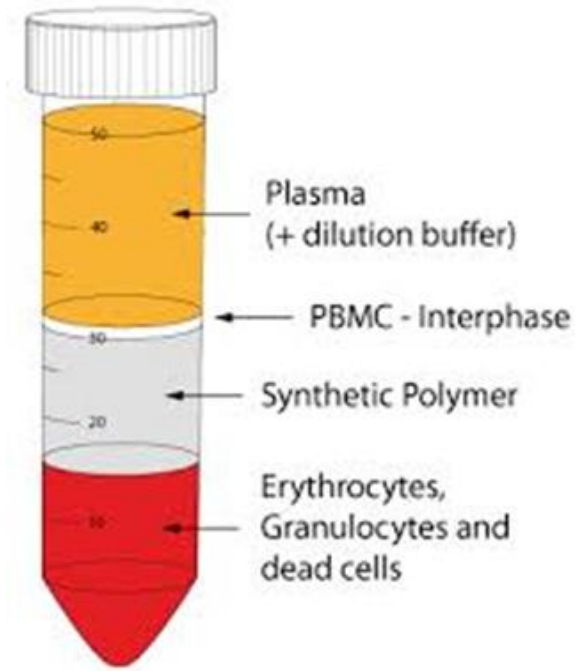
MAT testing at NoMA



MAT – Isolation of PBMC

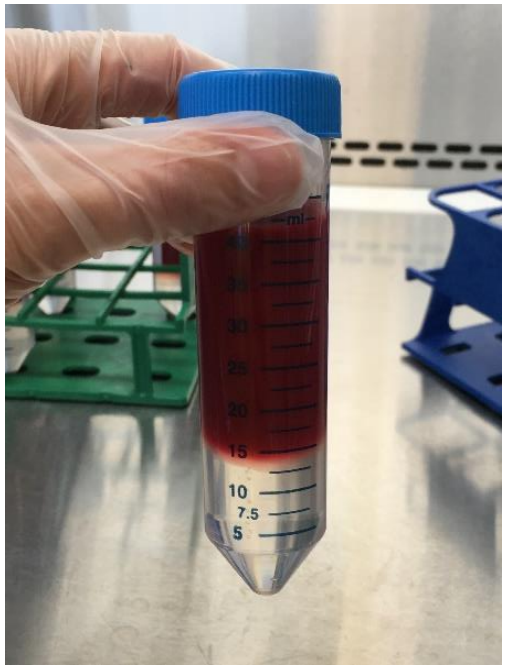


- Whole blood collected w/CPD from 4 donors
- Leukocyte bags produced by centrifugation

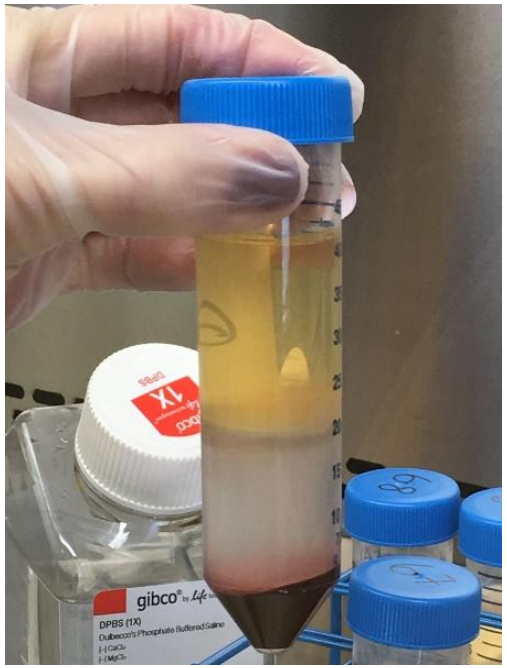


- Density gradient centrifugation w/Histopaque
- Peripheral blood mononuclear cells (PBMC) interphase collected

MAT – Isolation of PBMC



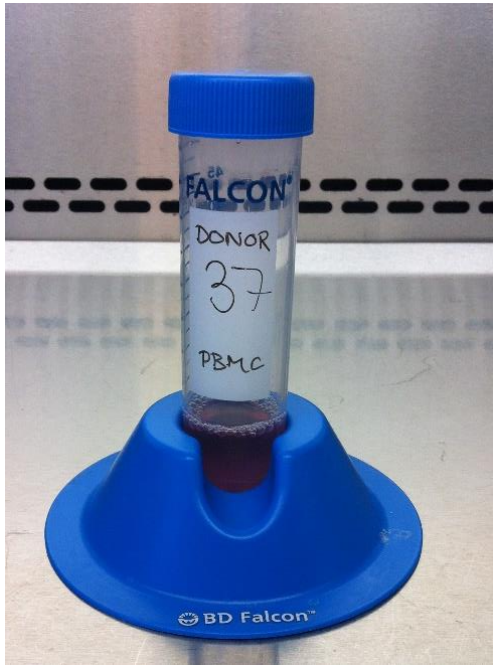
- Transfer 10-20 ml leukocytes to 50 ml tube
- Diluted to 50 ml in PBS
- Layer over Histopaque
- Centrifuge, 800 g, 30 min



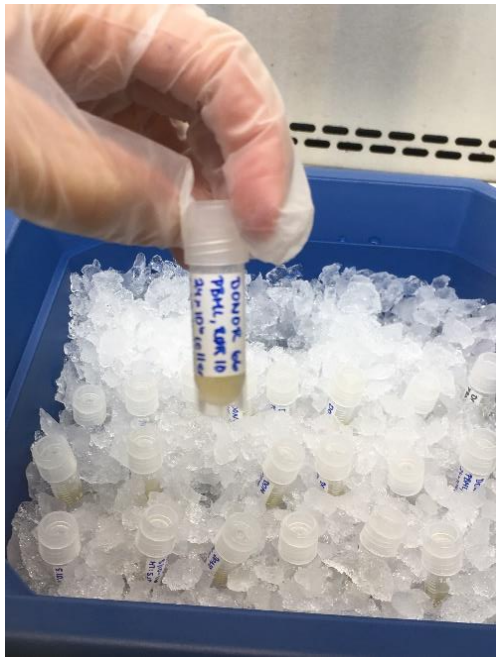
- Remove plasma
- Transfer PBMC new tube
- Add PBS and mix
- Centrifuge, 310 g, 10 min

- Remove supernatant
- Transfer PBMC new tube
- Wash in PBS
- Centrifuge, 310 g, 10 min

MAT – Isolation and freezing of PBMC



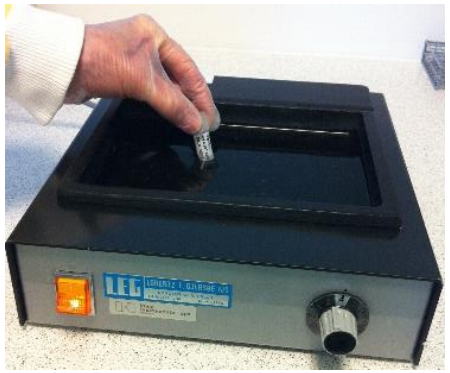
- Resuspend PBMC in RPMI-c
- Count cells at 1/20 dilution
- Centrifuge, 310 g, 10 min



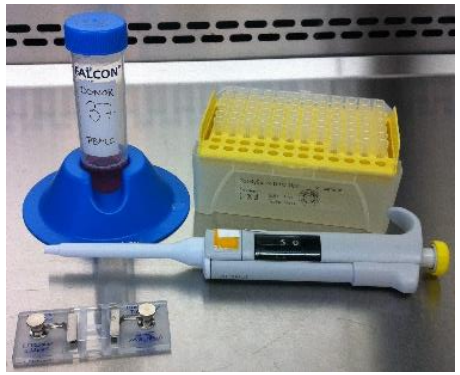
- Resuspend in freezing medium (DMSO/Human Serum)
- 24x10^6 cells/vial
- 1,2 ml cells/vial

- Freeze at -70°C, 3-5 days
- Store LN up to 2 years
- 200-500x10^6 cells/donor
- 8-20 vials/donor
- 3 batches tested/vial

MAT – Pyrogen stimulation of PBMC

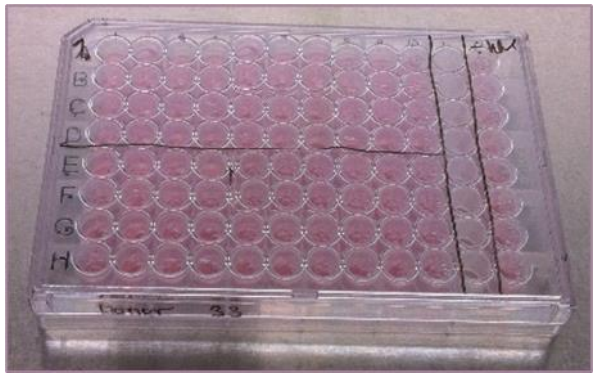


- Thaw PBMC
- Wash cells x2 in PBS



- Resuspend in RPMI-c
- Count cells, 1/5 dilution
- Dilute cells to $1x10^6$ cells/ml

Add PBMCs to plate

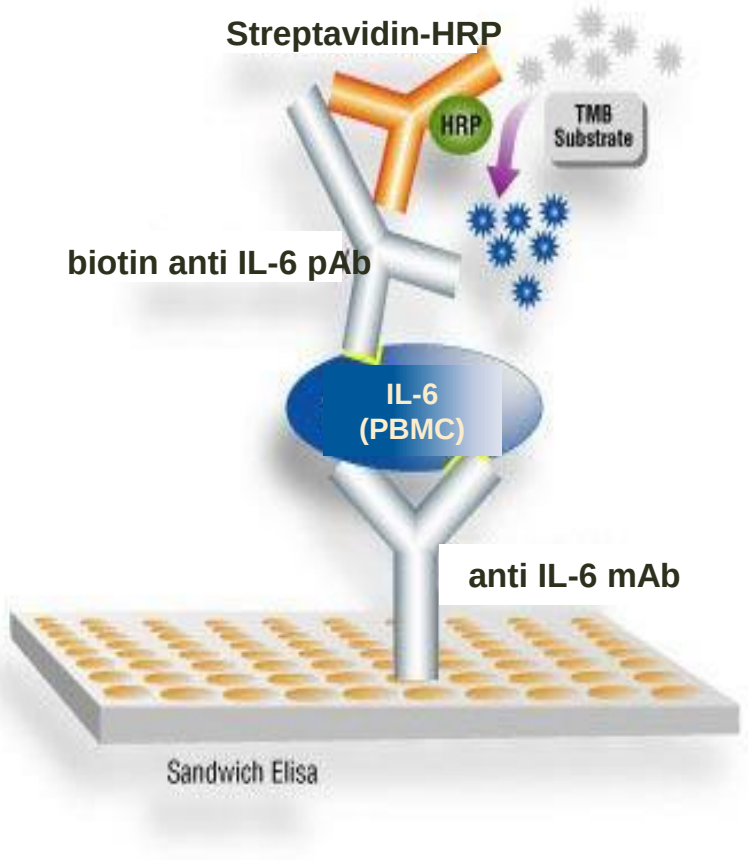


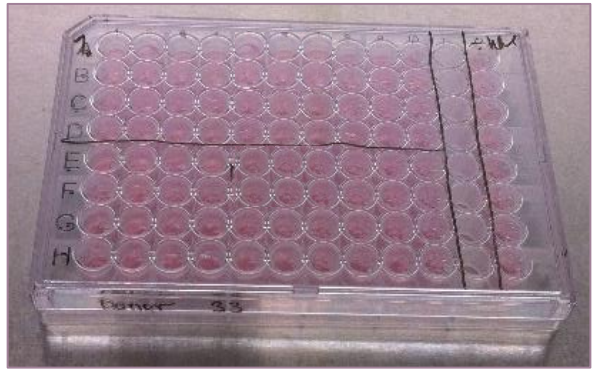
- $1x10^5$ cells/well
- Incubate, 37°C, 5% CO_2
- 18-22 hrs

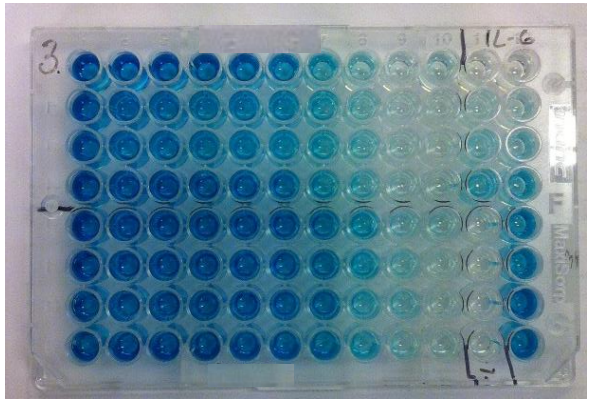
- Vaccine 2-fold serial dilution in RPMI-c

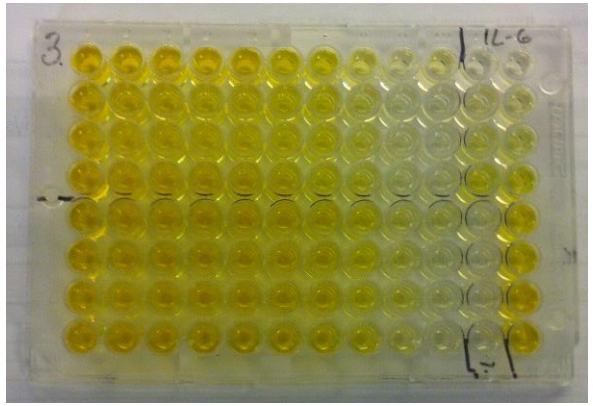
Add diluted vaccine to plate

MAT – ELISA


Streptavidin-HRP
HRP
TMB Substrate
biotin anti IL-6 pAb
IL-6 (PBMC)
anti IL-6 mAb
Sandwich Elisa




3.
IL-6

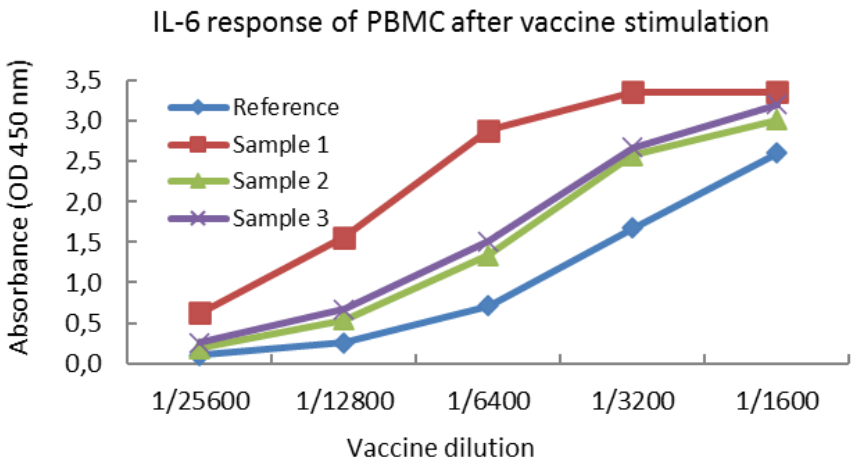

3.
IL-6

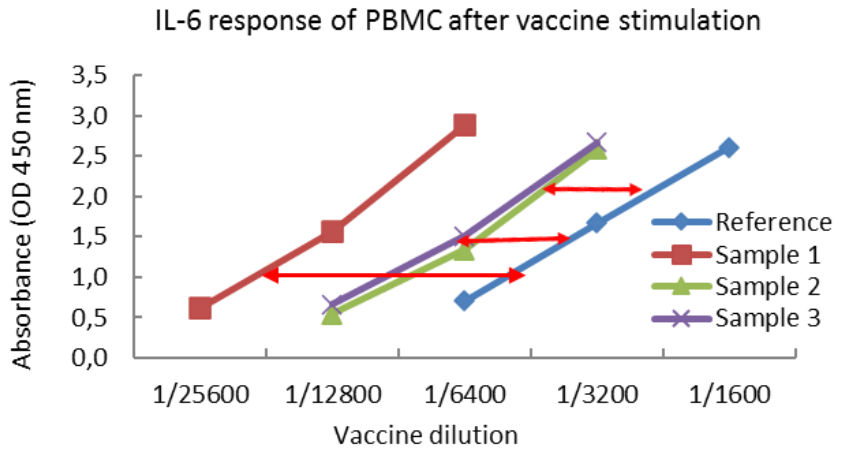
MAT – Data analysis

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Ref-D1	Ref-D2	Ref-D3	Ref-D4	Ref-D5	S1-D1	S1-D2	S1-D3	S1-D4	S1-D5	62,5
B	Blank	Ref-D1	Ref-D2	Ref-D3	Ref-D4	Ref-D5	S1-D1	S1-D2	S1-D3	S1-D4	S1-D5	250
C	Blank	Ref-D1	Ref-D2	Ref-D3	Ref-D4	Ref-D5	S1-D1	S1-D2	S1-D3	S1-D4	S1-D5	1000
D	Blank	Ref-D1	Ref-D2	Ref-D3	Ref-D4	Ref-D5	S1-D1	S1-D2	S1-D3	S1-D4	S1-D5	4000
E	-ctrl.	S2-D1	S2-D2	S2-D3	S2-D4	S2-D5	S3-D1	S3-D2	S3-D3	S3-D4	S3-D5	62,5
F	-ctrl.	S2-D1	S2-D2	S2-D3	S2-D4	S2-D5	S3-D1	S3-D2	S3-D3	S3-D4	S3-D5	250
G	-ctrl.	S2-D1	S2-D2	S2-D3	S2-D4	S2-D5	S3-D1	S3-D2	S3-D3	S3-D4	S3-D5	1000
H	-ctrl.	S2-D1	S2-D2	S2-D3	S2-D4	S2-D5	S3-D1	S3-D2	S3-D3	S3-D4	S3-D5	4000

ELISA Plate Layout
Blank: RPMI-c medium
Neg. ctrl.: PBMCs
IL-6 Standard (pg/ml)
Ref: Reference batch
S: Sample batch
D: Dilution
Possible Endotoxin Standard

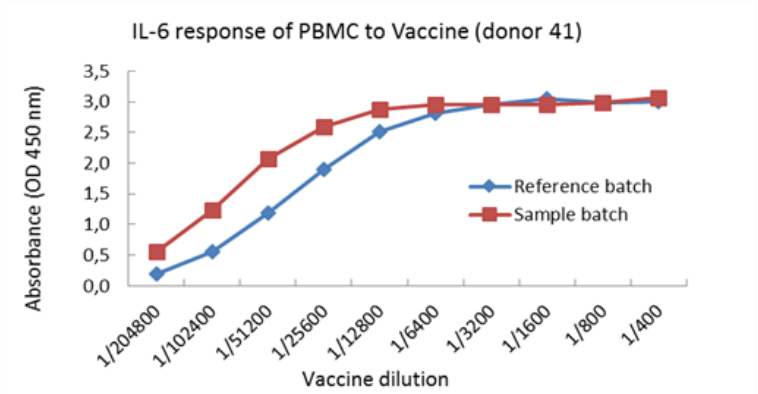
Absorbance is measured at 450 nm

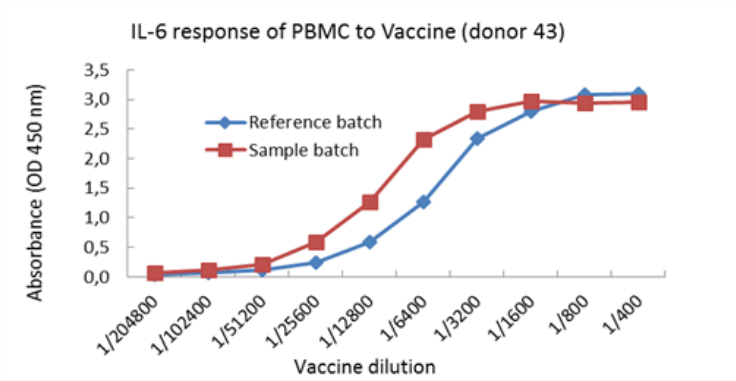


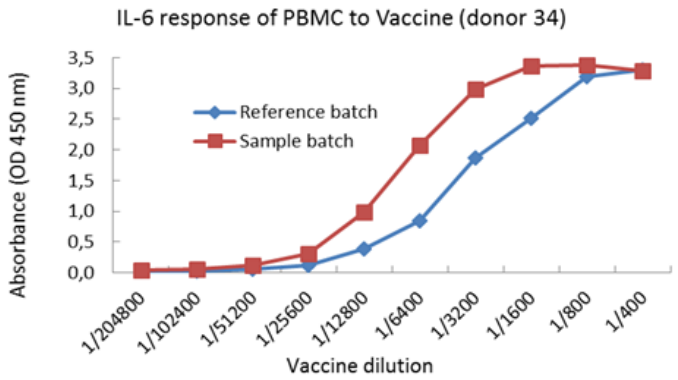


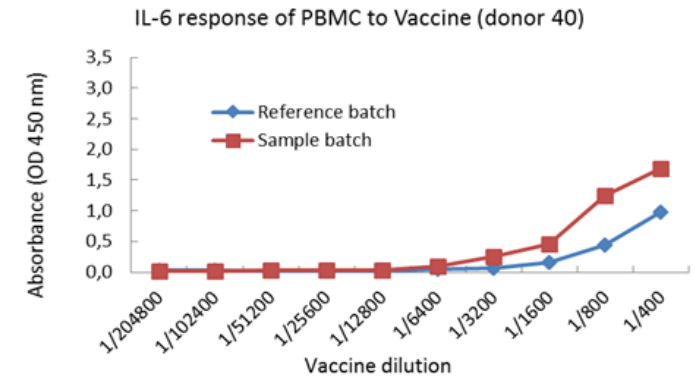
Donor to donor variability

- **Donor variation - biggest contributor to test variability**
- Donor qualification assay
  - Every donor tested in one assay with 10 dilutions of reference vaccine and test vaccine
  - Optimal vaccine dilution range varies from donor to donor
- PBMC stability – 24 months







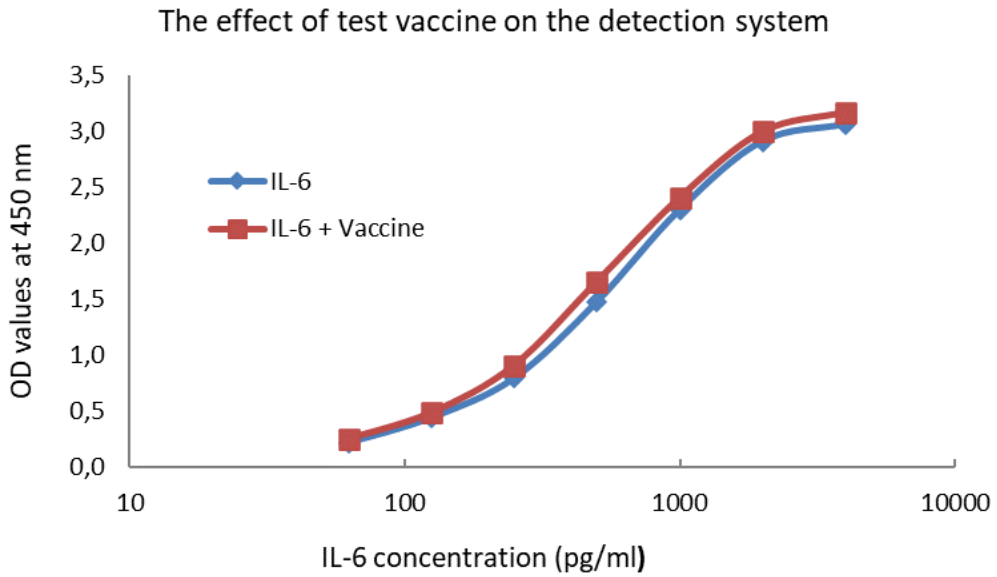


Product specific validation - ICH Q2 (R1)

- Specificity
- Linearity
- Range
- Accuracy
- Precision
- Limit of Detection – N/A
- Limit of Quantification – N/A
- Robustness
  - Incubation time
  - Cell concentration
  - Stability of frozen PBMC

Specificity

- Ph Eur MAT monograph requires that the product does not interfere with the detection method.
- ODs for the IL-6 curves in the presence and absence of test vaccine should not differ by more than 20%
- The test vaccine does not interfere with the detection of IL-6

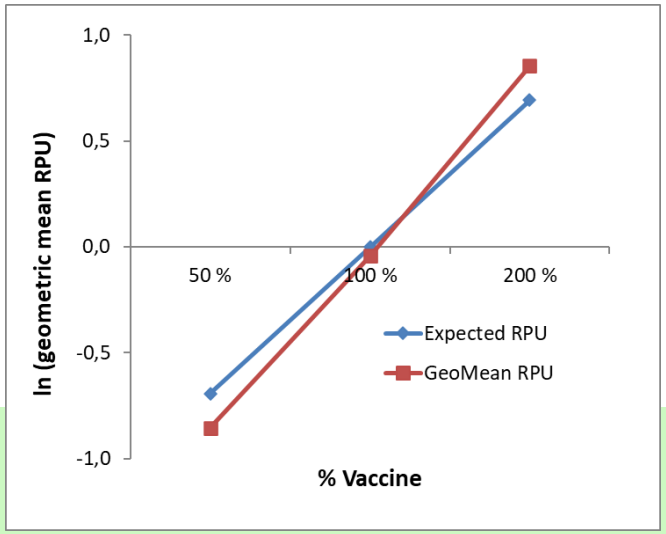


Accuracy

- *The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.*
- Vaccine formulated to contain 200% of each antigen, keeping the Aluminum hydroxide concentration constant. Vaccine batch tested in a 200%, 100% and 50% dilution and the 100% dilution was used as reference.
- Test performed using PBMCs from 20 different donors

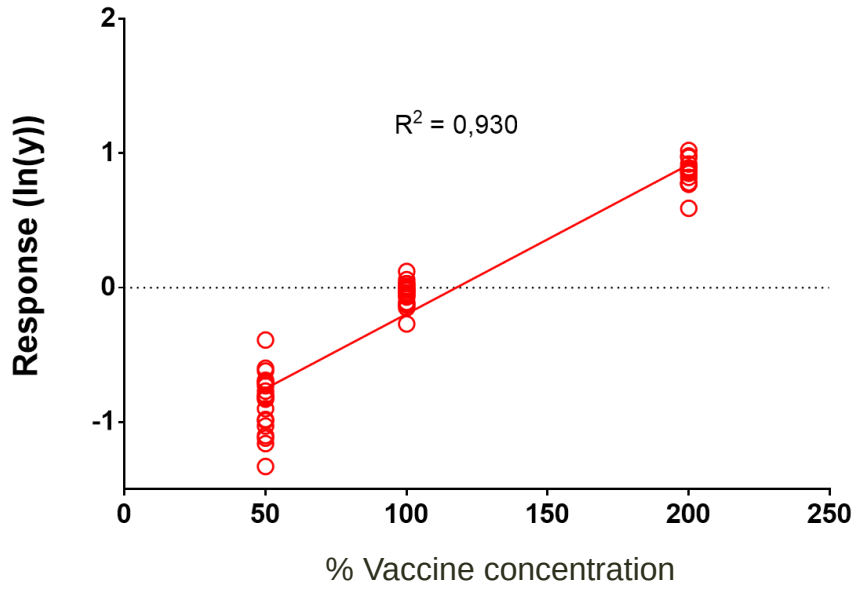
- **Validation criteria**
  For each level of concentration tested, it is required that the:
  - 95% upper confidence interval of [ln(RPU) measured – ln (RPU) target] calculated must be ≤ 0.30
  - 95% lower confidence interval of [ln(RPU) measured – ln (RPU) target] calculated must be ≥ -0.30
- **Results**

	Vaccine Concentration		
	200%	**100%**	**50%**
95% UCL [ln(RPU) measured – ln (RPU) target]	0.21	0.00	-0.06
95% LCL [ln(RPU) measured – ln (RPU) target]	0.11	-0.08	-0.27



Linearity

- Linearity is part of the assay validity criteria for each test
  - Regression p-value ≤ 0.01
  - Non-linearity p-value ≥ 0.01
  - Non-parallelism p-value ≥ 0.01
- In every MAT, the reference batch and each test batch is analyzed in five serial dilutions
- Three dilutions giving the steepest slope used for further analysis
- The assay validity criteria requires that for each range of three dilutions, the p-value for non-linearity must be non-significant (p ≥ 0.01)
- Accuracy studies confirmed that there was no evidence of non-linearity and there was a significant regression as sample concentration decreased.



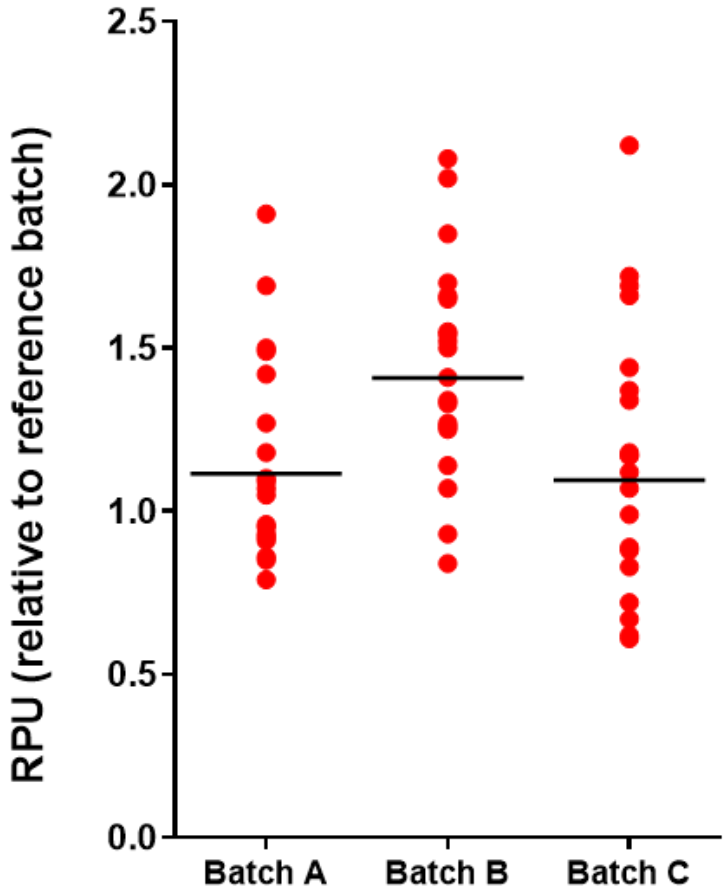
Range

- Through accuracy testing of a vaccine containg 50% - 200% antigen, the MAT was shown to be linear in the range from 50% to 200%

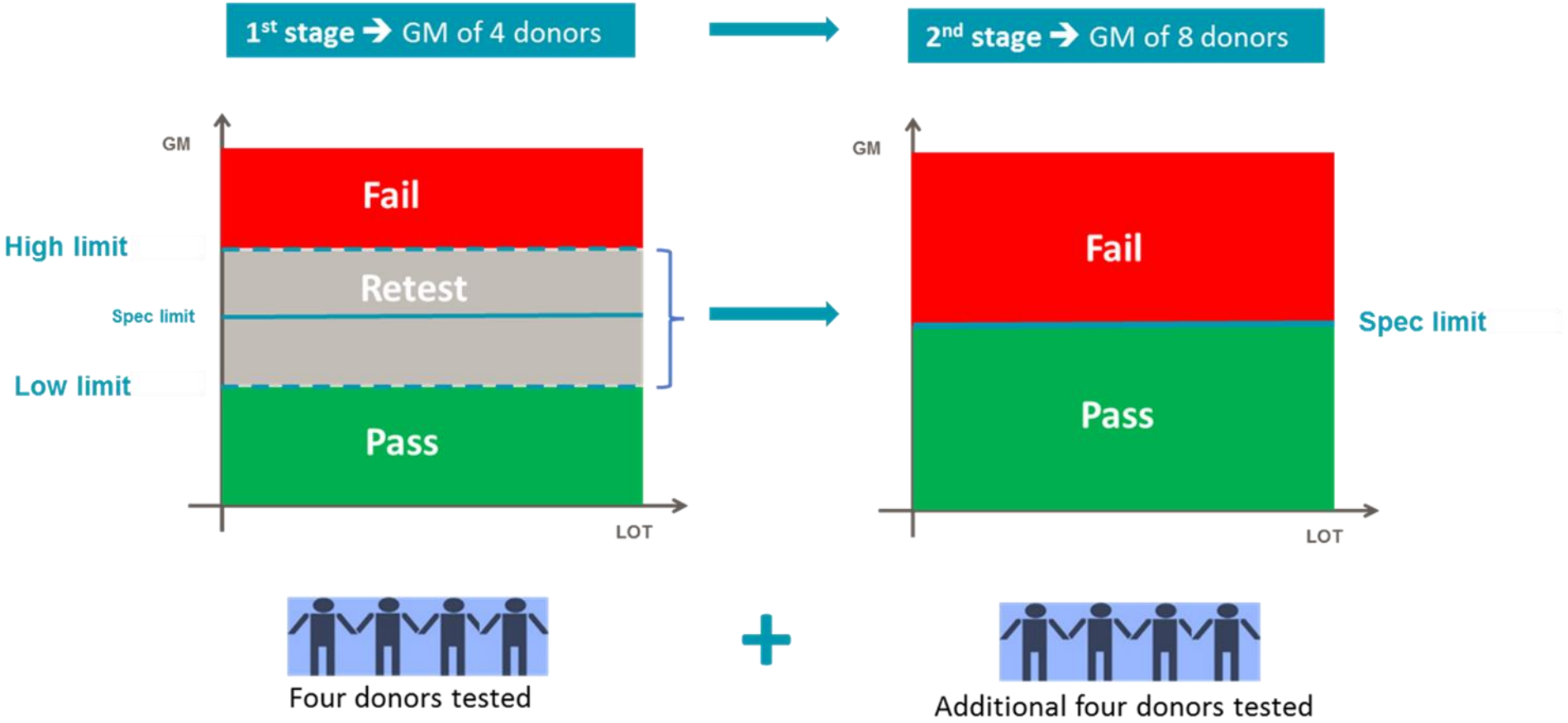
Precision

- 3 different vaccine batches were tested in 21 donors on 3 different days
- The largest contributor to test variability is donor to donor variation, giving a high GCV%

	Batch A	Batch B	Batch C
GeoMean RPU	1.12	1.41	1.10
n	21	20	20
GCV%	27.7%	26.8%	43.1%

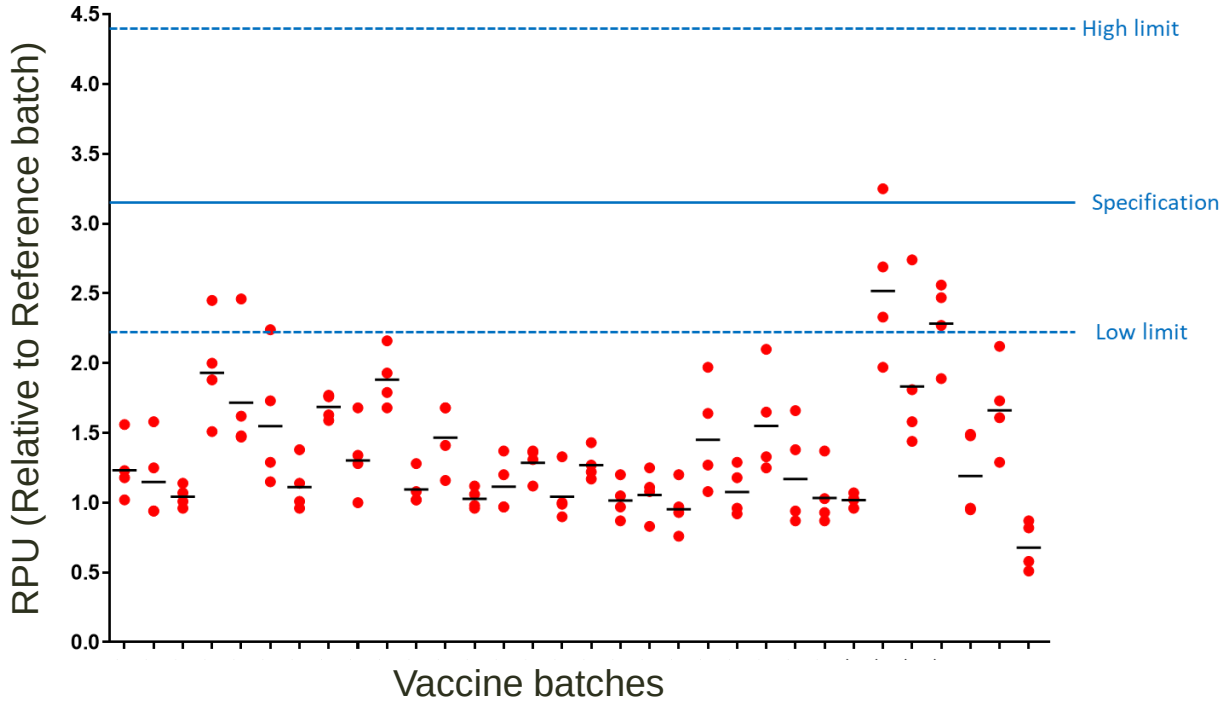


MAT Specification

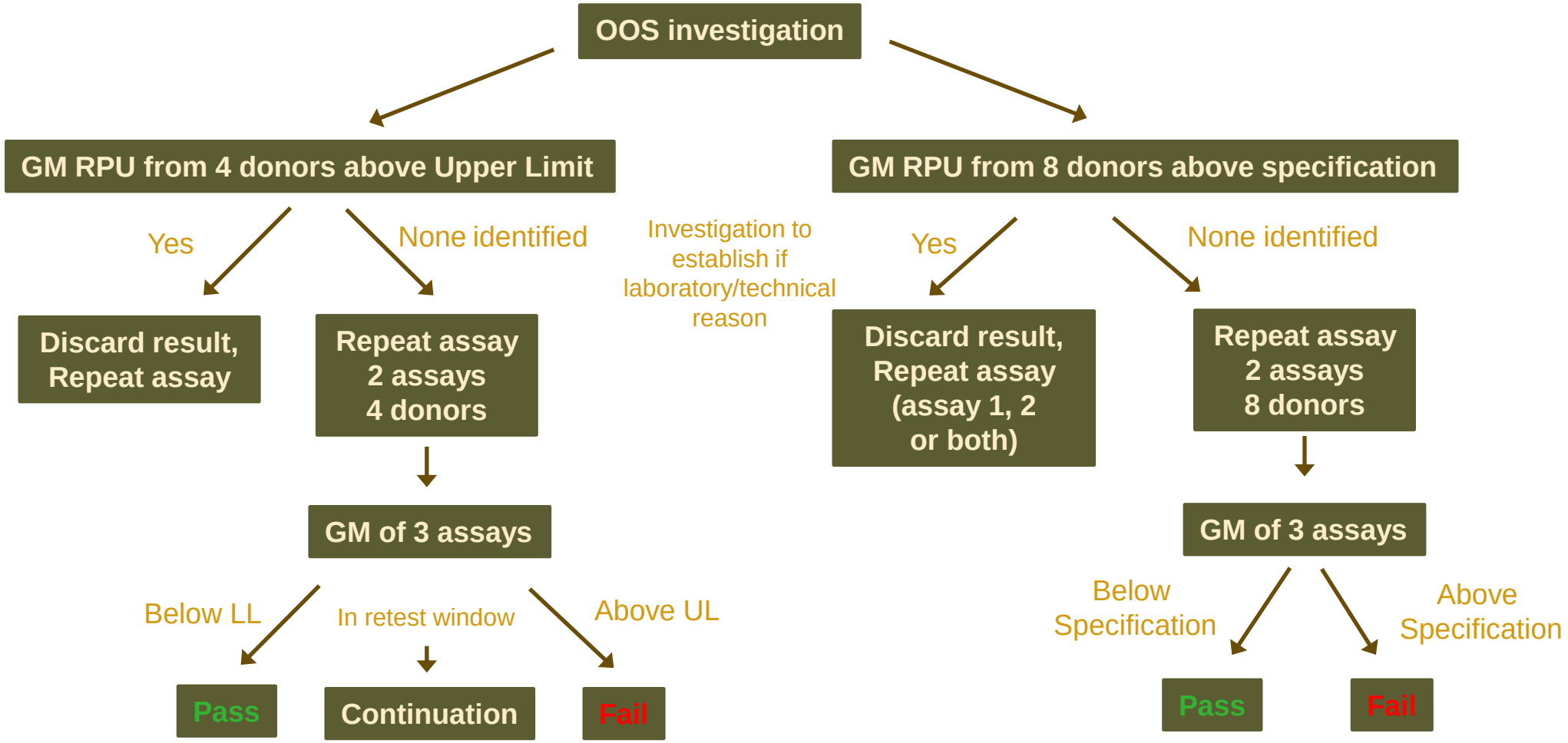


Setting a specification limit

- Specification settings were calculated using:
  - **one-sided tolerance interval, with a 99% population coverage and 95% confidence**
- 32 different vaccine batches tested in 4 donors each



OOS Procedure



2.6.40. Monocyte-activation test for vaccines containing inherently pyrogenic components

- General chapter describing the use of MAT to test vaccines containing inherently pyrogenic compounds (e.g. OMV, lipidated proteins)
- Monitor the consistency of pyrogen levels when the pyrogens are an integral part of the vaccine
- Use an in-house reference lot of a safe and efficacious vaccine
- 2.6.40 to be used in conjunction with general chapter 2.6.30
- Published in Ph. Eur. In January 2024
- Implemented on July 1st 2024

dmp.no

helsenorge.no

Direktoratet for medisinske produkter

Norwegian Medical Products Agency