Use of different serum sources in testing human Albumin preparations

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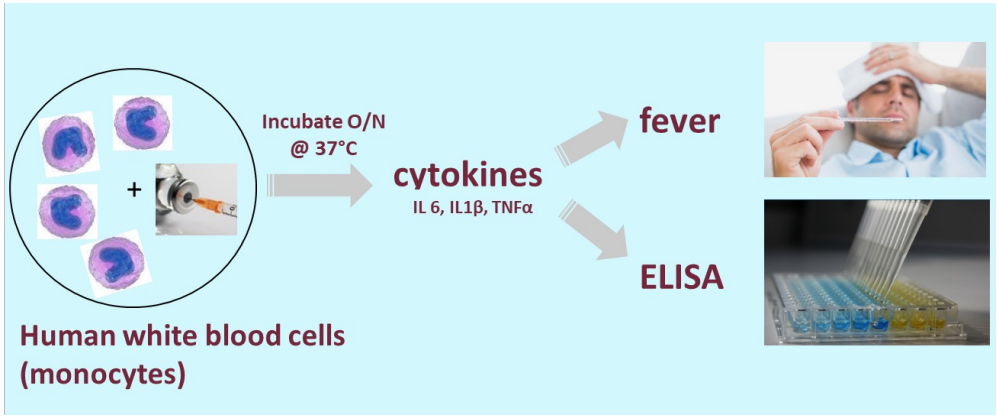
Different MAT formats

- Monocyte sources
  - Whole blood
  - Fresh PBMCs
  - Cryopreserved PBMCs
  - Cell lines



- Genetic variation coverage: Single vs pooled PBMCs

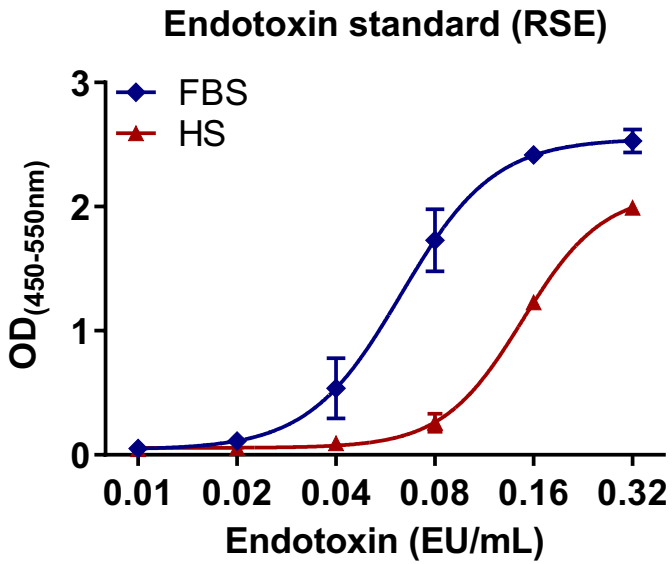
- Read out formats
  - IL-6 ELISA
  - IL-1β
  - TNF-α
  - Cell lines with reporter genes, NFkB-luciferase

Our MAT set-up

- Cryopreserved pooled PBMCs from 4 donors
  - Available on demand
  - Includes donor variation
  - Control reactogenicity towards specific pyrogens
  - Stable (months at -80°C, years in liquid N_2)
- Different serum sources
  - FBS
  - HS
- IL-6 ELISA
  - High sensitivity
  - Good correlation with fever

Kit: MAT Cell Set from Essange Reagents / PyroCell from Lonza

Effect of different serum sources in MAT: Endotoxin



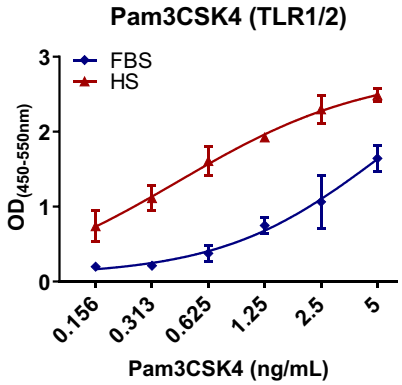
Serum source	sensitivity (EU/mL)
FBS	≤ 0.02
HS	≤ 0.08

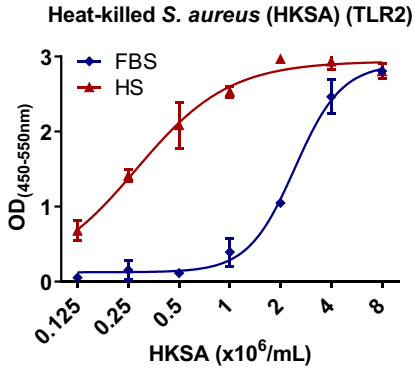
→ Lower reactivity to endotoxin with Human Serum (HS) than with Fetal Bovine Serum (FBS)

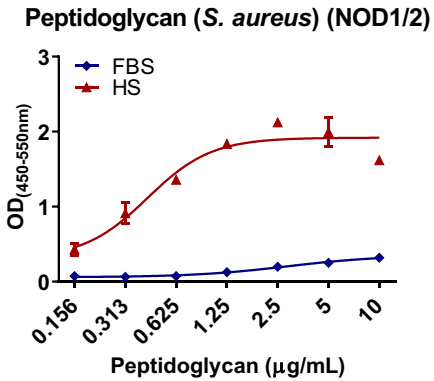
Molenaar-de backer et al. *Altex* 2021
https://doi.org/10.14573/altex.2008261

Effect of different serum sources in MAT: NEPs

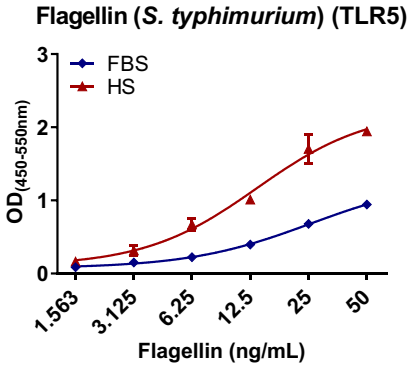
Higher reactivity with HS

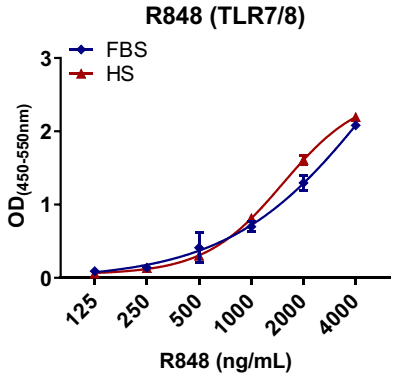






Comparable reactivity





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Conclusion effect of serum source

- Use of HS results higher reactivity towards most tested NEPs
  - Pam3CSK4 → Higher
  - HKSA → Higher
  - Peptidoglycan → Higher
  - Flagellin → No significant difference
  - R848 → No significant difference

- Use of HS results in lower reactivity towards endotoxin compared to FBS
  - Higher test sensitivity

    → lower Maximum valid dilution (MVD)

$$\textbf{MVD=} \frac{CLC \times C}{Test\ sensitivity}$$

Why testing Albumins with MAT?

- FBS based MAT in 2015
- Albumin preparations can contain NEPs
  ⟶ BET not appropriate

Table 4. Testing the pyrogenicity of different batches of human albumin.

	BET	RPT	MAT
Batch 1	–	–	–
Batch 2	–	+	+
Batch 3	–	+	+
Batch 4	–	+	+

Four albumin batches were tested, of which some showed different outcomes in the RPT and the BET. As seen in this table, the MAT and the RPT gave identical results. However, batches #2, #3 and #4, which were pyrogenic and induced IL-6 in the MAT, passed the BET.

Solati S. et al. *Innate Immunity*, 2015
https://doi.org/10.1177/1753425915583365

MAT and 20% Albumin: spiking endotoxin

FBS based MAT

- MVD = 1.3/ 0.02 = 65x per sample (= 130x per well)

Batch	Dilution per well	Endotoxin recovery	Final result (EE/mL)
1	12	176	0.212
	24	136	
	48	107	
2	12	169	0.202
	24	135	
	48	130	
3	12	103	0.254
	24	96	
	48	90	

HS based MAT

- MVD = 1.3/ 0.06 = 21x per sample (=42x per well)

Batch	Dilution per well	Endotoxin recovery	Final result (EE/mL)
1	10	***	
	20	189	0.408
	40	170	
2	10	123	<0.324
	20	108	
	40	101	
3	10	107	<0.324
	20	108	
	40	113	

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Conclusions

- HS based MAT lower sensitivity towards endotoxin, but higher sensitivity towards multiple NEPs.
- HS based MAT better reflects human immune system and animal friendlier then FBS based MAT.
- In MAT testing of albumin preparations both serum sources can be used. For other PDMPs this can be different

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