The Cuban Experience with the MAT Pyrogenicity Test on Parenteral Human Serum Albumin

Rolando Perdomo-Morales

Center for Pharmaceuticals Research and Development/

Centro de Investigacion y Desarrollo de Medicamentos (CIDEM)

Havana, Cuba

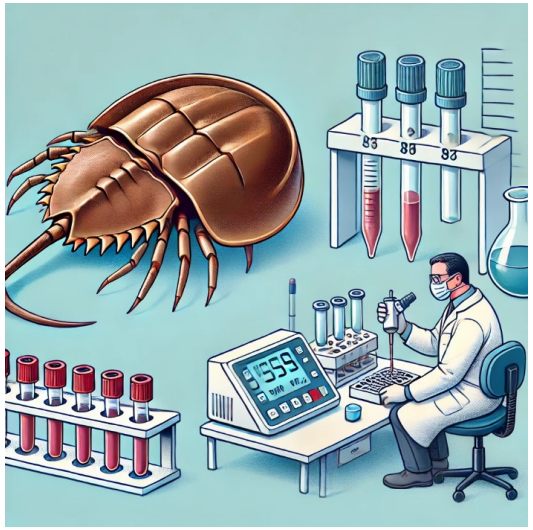
Paul Ehrlich Institute-CIDEM

Perdomo-Morales R, Pardo-Ruiz Z, Spreitzer I, Lagarto A, Montag T. Monocyte activation test (MAT) reliably detects pyrogens in parenteral formulations of human serum albumin. ALTEX. 2011;28(3):227-35.

Why testing Albumin?

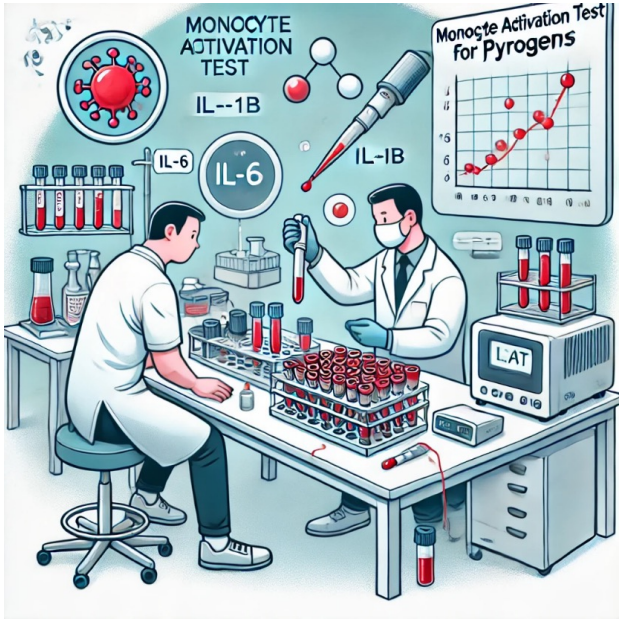
- We had access to blood products manufactured in Cuba (pyrogenic and non-pyrogenics by RPT). There is still interest in implementing MAT for safety reasons.
- Albumin can bind LPS. It is probably the first relevant example of protein masking of LPS.
- There were numerous scientific reports related to the pyrogenicity of albumin (humans vs. rabbits, rabbits vs. LAL, MAT, etc.). Conflicting results!
- At that time, the main Pharmacopoeias (except the JP) indicated the RPT for evaluating pyrogens in parenteral human albumin.
- Albumin is one of the best-selling biopharmaceutical products worldwide

Aim of the study



Kinetic chromogenic LAL from ACC (Pyrochrome).

Sixteen lots of 20% HSA were evaluated with the conventional and endotoxin-specific LAL/BET, the rabbit pyrogen test (RPT), and the monocyte activation test (MAT) using fresh human whole blood and IL-6 and IL-1β as readouts.



Batch	Dilution	C-LAL		Dilution	ES-LAL	
		Concentration (EU/ml)[a]	Spike recovery (%)		Concentration (EU/ml)[a]	Spike recovery (%)
A	10	23.46 ± 0.709	52.2	10	0.05 ± 0.006	52.4
	100	0.51± 0.021	158.0	100	< 0.01	39.4
	300	0.16 ± 0.002	170.2	400	< 0.01	41.8
	500	0.12 ± 0.009	121.4			
B	10	2.33 ± 0.069	230.6	10	0.06 ± 0.003	32.6
	100	0.15 ± 0.011	75.1	100	< 0.01	37.2
	300	0.09 ± 0.004	62.7	400	< 0.01	38.4
	500	0.05 ± 0.001	52.4			
D	10	1.27 ± 0.025	147.7	10	0.05 ± 0.002	70.8
	100	0.11 ± 0.004	132.7	100	< 0.01	41.6
	300	0.06 ± 0.004	175.1	400	< 0.01	40.8
	500	0.04 ± 0.003	82.2			
F	10	2.33± 0.105	164.1	10	0.08 ± 0.006	49.0
	100	0.19 ± 0.004	103.0	100	< 0.01	39.7
	300	0.08 ± 0.001	78.0	400	< 0.01	35.3
	500	0.05 ± 0.004	129.4			
G	10	0.63 ± 0.000	206.9	10	0.14 ± 0.000	69.5
	100	0.05 ± 0.004	107.0	100	< 0.01	50.9
	300	0.03 ± 0.001	75.9	400	< 0.01	56.9
	500	0.08 ± 0.003	99.9			
H	10	4.62 ± 0.000	136.8	10	1.74 ± 0.064	55.2
	100	0.25 ± 0.000	99.0	100	0.13 ± 0.012	56.8
	300	0.07 ± 0.004	93.6	400	< 0.01	57.7
	500	0.04 ± 0.001	82.6			
I	10	3.10 ± 0.037	529.0	10	0.41 ± 0.019	77.6
	100	0.13 ± 0.001	109.8	100	0.04 ± 0.01	53.4
	300	0.03 ± 0.001	90.2	400	< 0.01	59.8
	500	0.03 ± 0.001	81.6			
J	10	1.95 ± 0.046	213.0	10	0.11 ± 0.008	41.2
	100	0.09 ± 0.004	81.6	100	< 0.01	40.2
	300	0.04 ± 0.001	57.5	400	< 0.01	37.1
	500	0.02 ± 0.001	81.1			
K	10	3.21 ± 0.002	255.0	10	0.13 ± 0.004	38.8
	100	0.26 ± 0.011	189.8	100	< 0.01	44.0
	300	0.09 ± 0.006	105.0	400	< 0.01	48.3
	500	0.06 ± 0.004	99.9			
P	10	5.30 ± 0.156	100.8	10	0.19 ± 0.006	36.1
	100	0.17 ± 0.002	81.5	100	< 0.01	36.0
	300	0.06 ± 0.001	52.3	400	< 0.01	40.1
	500	0.03 ± 0.001	51.0			

Interference test of 20 % HSA in Conventional and Endotoxin-Specific LAL Assay.

All HSA batches were contaminated with (1,3)-β-glucans as shown by the differences found between conventional and endotoxin-specific LAL assay. The main source of contaminating (1,3)-β-glucans in blood-derived products are cellulose membranes commonly used for clarification.

The spikes were recovered only in batches that failed the rabbit pyrogen test!!

*Hochstein et al. (1979) suggested that HSA masks certain amounts of endotoxin until **saturation** occurs, so the LAL test is not able to fully detect the endotoxin spikes.*

Hochstein HD et al. Limulus amebocyte lysate testing of nomral serum albumin (human) in the United States since 1975. Dev Biol Stand. **1979**;44:35-42.

BET/LAL Partial Conclusions

- All 20 % HSA batches were contaminated with significant amounts of 1,3-β-glucans, which could go unnoticed by the typical enhancing reaction to endotoxin spikes, especially when the endotoxin detected by LAL is very low. Possible source of errors or false positives.

- Albumin may mask endotoxins to the LAL reagent, leading to failures in the recovery of the positive product controls or spikes. The estimated stoichiometry of albumin and LPS binding (HSA:LPS) is 1:10 molar (HSA saturation) (1).

- The LAL test is not suitable for assessing endotoxin content in HSA due to interference problems (hot spike recovery less than 50%).

Jürgens G, Müller M, Garidel P, Koch MH, Nakakubo H, Blume A, Brandenburg K. Investigation into the interaction of recombinant human serum albumin with Re-lipopolysaccharide and lipid A. J Endotoxin Res. 2002;8(2):115-26.

Interference test of 20 % HSA in WB-MAT using IL-6 and IL-β as readouts.

Endotoxin recoveries (%)

Readout	Batch	Dilutions					
		1/2	1/4	1/8	1/16	1/32	1/64
IL-1β	A	169.8	94.3	133.4	106.4	80.5	76.0
	B	117.8	160.7	158.4	90.9	83.8	92.0
	C	12.2	145.7	149.7	120.9	76.6	58.0
	D	70.0	72.2	98.9	148.1	132.6	109.9
	F	66.8	101.2	96.2	78.9	69.4	75.0
IL-6	A	N.D.	N.D.	> 200	> 200	67.7	112.7
	B	N.D.	N.D.	> 200	> 200	129.0	125.5
	C	N.D.	N.D.	> 200	97.4	38.6	154.0
	D	N.D.	N.D.	> 200	> 200	> 200	185.1
	F	N.D.	N.D.	> 200	> 200	157.9	133.3

MVD (IL-1β): 1/32
MVD (IL-6): 1/64

MinVD (IL-1β): 1/4
MinVD (IL-6): 1/32

- (1,3)-β-glucans from filters induced an IL-6 enhanced response to endoxins.
- No masking effect was observed for the hot spike using IL-1β readout.
- IL-1β appears to be a better readout when samples are contaminated with glucans from cellulose-based filters.

Comparative results of pyrogen evaluation by MAT, RPT and Endotoxin-Specific LAL in 20 % HSA

Batch	MAT (IL-1β) (EEU/ml)[a]	MAT (IL-6) (EEU/ml)[a]	RPT (∑ ΔT) (°C)	ES-LAL (EU/ml)[a]
A	0.81 ± 0.242[P]	< 2[P]	0.60[P]	0.52 ± 0.003[P]
B	0.93 ± 0.224[P]	2.22 ± 0.921[P]	0.05[P]	0.61 ± 0.029[P]
C	1.29 ± 0.245[P]	5.10 ± 0.183[F]	0.10[P]	1.30 ± 0.038[P]
D	1.29 ± 0.406[P]	2.52 ± 0.760[P]	0.25[P]	0.54 ± 0.017[P]
E	1.15 ± 0.364[P]	2.83 ± 0.609[P]	0.35[P]	0.64 ± 0.077[P]
F	1.07 ± 0.517[P]	2.62 ± 1.084[P]	0.35[P]	0.82 ± 0.062[P]
G	>8[F]	8.64 ± 1.28[F]	5.30 (first retest)[F]	1.39 ± 0.000[P]
H	>8[F]	14.67 ± 4.31[F]	5.70 (first retest)[F]	15.35 ± 2.480[F]
I	3.57 ± 0.795[P]	3.85 ± 0.75[P]	7.05 (third retest)[F]	4.07 ± 0.187[F]
J	< 0.5[P]	< 2[P]	0.55[P]	1.09 ± 0.077[P]
K	< 0.5[P]	< 2[P]	0.50[P]	1.33 ± 0.039[P]
L	< 0.5[P]	2.35 ± 0.022[P]	1.85 (first retest)[P]	1.03 ± 0.025[P]
M	1.04 ± 0.157[P]	< 2[P]	0.55[P]	0.96 ± 0.035[P]
N	0.68 ± 0.182[P]	2.36 ± 1.006[P]	0.20[P]	0.72 ± 0.000[P]
O	0.80 ± 0.233[P]	2.72 ± 0.985[P]	0.15[P]	1.43 ± 0.048[P]
P	1.48 ± 0.280[P]	< 2[P]	0.35[P]	1.93 ± 0.063[P]

The concentrations in Batch I were around the limit (4 EU/ml) for MAT/IL-6, MAT/IL-1β and LAL tests!

The endotoxin equivalent concentrations estimated using the IL-6 readout were generally higher than those obtained with IL-1β.

Batch C failed the MAT/IL-6 test but passed the MAT/IL-1β, RPT, and LAL tests.

Two batches failed the MAT (IL-6 and IL-1β) and RPT, while one of them passed the LAL test.

A third batch (I) failed both LAL and RPT tests but passed the MAT/IL-6 and MAT/IL-1β tests.

RPT is no longer mandatory for testing albumin according to the USP and EP.

According to USP:
Albumin Human conforms to the regulations of the federal Food and Drug Administration concerning biologics (640.80 to 640.86) (see *Biologics* <1041>).

<1041> BIOLOGICS
……. and see *Safety Tests—Biologicals* under *Biological Reactivity Tests,…*and *Pyrogen Test* < 151 >, as well as *Bacterial Endotoxins Test* < 85>)...

According to EP (11.6)
Pyrogens (2.6.8) **or Bacterial endotoxins** (2.6.14)
It complies with the test for pyrogens or, preferably and where justified and authorised, with a validated *in vitro* test such as the bacterial endotoxin test.

Why considering MAT as a better choice for testing Albumin for pyrogens?

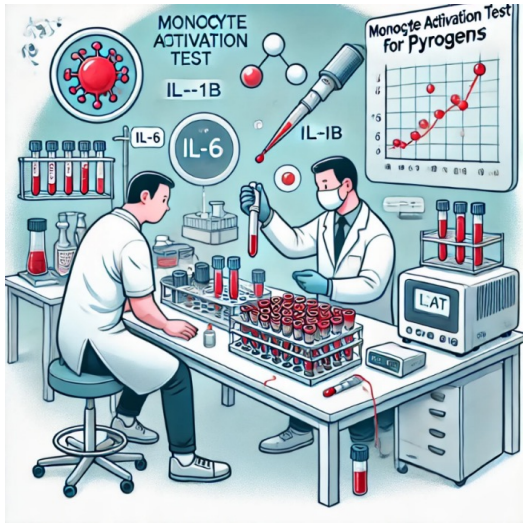
1. Resembles better the human fever reaction.

2. Albumin binds to endotoxins!!
   - Some authors include albumin as part of the LPS-binding proteins (LBP) similar to HDL and other proteins.
   - Albumin's ability to bind LPS has been used for endotoxin removal (MATISSE–Fresenius system).
   - Albumin presents endotoxins to the immune system more efficiently, inducing a more significant response to the same concentration of free LPS.

3. Albumin's biological half-life is 19 days (it lasts 16-18 hours in circulation) in a healthy person. Contaminated albumin could pose a challenging scenario when administered parenterally (e.g. liver cirrhosis).

4. Endotoxin bound to albumin can be readily detected by WB/MAT-IL-β, but masked to LAL

THANKS



The Team!

Paul Ehrlich Institute:

Dr. Ingo Spreitzer
Dr. Thomas Montag

CIDEM

Zenia Pardo-Ruiz
Vivian Montero-Alejo
Alicia Lagarto