Thurmann et al.:

Comparison of Pyrogen Assays by Testing Products Exhibiting Low Endotoxin Recovery

Supplementary Data

Tab. S1: Hold time spiked sample preparations for T0 and T3

	Product 1	Product 2	Product 3	LRW
mL 10,000 EU/mL RSE	0.560	0.132	0.150	0.560
mL sample	5.0	24.0	5.0	5.0
EU/mL	1,007	55	291	1,007

Tab. S2: Preparation of hold time samples for RPT using hold time preparations from Table S1

	Product 1	Product 2	Product 3	LRW
mL spiked hold-time sample	1.16	22.0	4.0	1.16
mL saline	99.0	81.0	96.0	99.0
EU/mL	11.7	11.7	11.7	11.7

Tab. S3: Pre-dilution of hold time samples for BET and MAT from Table S1

	Product 1	Product 2	Product 3	LRW
mL spiked hold-time sample	0.040	0.565	0.145	0.040
mL saline	2.0	1.0	2.0	2.0
EU/mL	19.7	19.7	19.7	19.7

Tab. S4: Preparation of samples for BET using pre-dilution preparations from Table S3

	Total fold dilution		
	10-fold	40-fold	400-fold (use 40-fold as pre-dilution)
Expected result (EU/mL)	2.0	0.5	0.05
Sample (mL)	0.100	0.200	0.100
LRW (mL)	0.900	0.600	0.900
Total volume (mL)	1.000	0.800	1.000

Tab. S5: Preparation of samples for MAT using 1.97 EU/mL predilutions from Table S3

	Total fold dilution					
	49-fold	66-fold	99-fold	131-fold	197-fold	263-fold
Expected result (EU/mL)	0.40	0.30	0.20	0.15	0.10	0.075
Sample (mL)	0.100	0.100	0.050	0.050	0.050	0.050
Kit-specific media (mL)	0.390	0.560	0.445	0.605	0.935	1.265
Total volume (mL)	0.490	0.660	0.495	0.655	0.985	1.315

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Tab. S6: Plate layouts

T0 Plate

	1–3	4–6	7–9	10–12
A	standard 1	Product 1: 49-fold dilution	Product 2: 49-fold dilution	Product 3: 49-fold dilution
B	standard 2	Product 1: 66-fold dilution	Product 2: 66-fold dilution	Product 3: 66-fold dilution
C	standard 3	Product 1: 99-fold dilution	Product 2: 99-fold dilution	Product 3: 99-fold dilution
D	standard 4	Product 1: 131-fold dilution	Product 2: 131-fold dilution	Product 3: 131-fold dilution
E	standard 5	Product 1: 197-fold dilution	Product 2: 197-fold dilution	Product 3: 197-fold dilution
F	standard 6	Product 1: 263-fold dilution	Product 2: 263-fold dilution	Product 3: 263-fold dilution
G	standard 7	LRW: 49-fold dilution	LRW: 99-fold dilution	LRW: 197-fold dilution
H	Blank	LRW:66-fold dilution	LRW: 131-fold dilution	LRW: 263-fold dilution

T3 Plate

	1–3	4–6	7–9	10–12
A	standard 1	Product 1: 49-fold dilution	Product 2: 49-fold dilution	Product 3: 49-fold dilution
B	standard 2	Product 1: 66-fold dilution	Product 2: 66-fold dilution	Product 3: 66-fold dilution
C	standard 3	Product 1: 99-fold dilution	Product 2: 99-fold dilution	Product 3: 99-fold dilution
D	standard 4	Product 1: 131-fold dilution	Product 2: 131-fold dilution	Product 3: 131-fold dilution
E	standard 5	Product 1: 197-fold dilution	Product 2: 197-fold dilution	Product 3: 197-fold dilution
F	standard 6	Product 1: 263-fold dilution	Product 2: 263-fold dilution	Product 3: 263-fold dilution
G	standard 7	LRW: 49-fold dilution	LRW: 99-fold dilution	LRW: 197-fold dilution
H	Blank	LRW:66-fold dilution	LRW: 131-fold dilution	LRW: 263-fold dilution

Figure S1 and S2 show the PBMC, IL-6 T0 and T3 response to RSE ranging from 0.0156 to 1.000 EU/mL RSE. The raw OD signals (average of 3 wells) were 0.5789 for the lower limit and 3.6581 for the upper limit. Samples had been serially diluted based on expected responses in spiked LRW controls using dilution factors from 49-263 (6 levels).

Separate curves for T0 and T3 were obtained to have samples at corresponding time points compared on each microplate. It is noted that the curve in Figure S2 is not as steep and left-shifted (vs that in Figure S1), illustrative of the variation between cell preparations for these types of assays. Replicates on both plates were very precise, as shown, and T0 has the expected curve shape with asymptotes on both the lower and upper concentration ranges. Variation in RSE EC50 responses between plates is not unusual for MAT assays and demonstrates the need for standardization within each plate for proper sample interpolation.

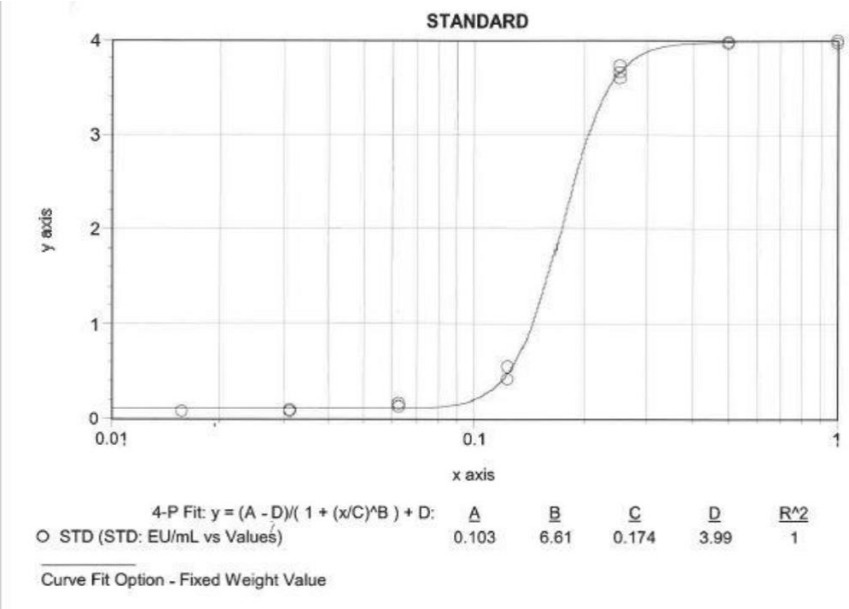

Fig. S1: IL-6 response of PBMCs to RSE standards at T0 (Y= 450nm, X = EU/mL)

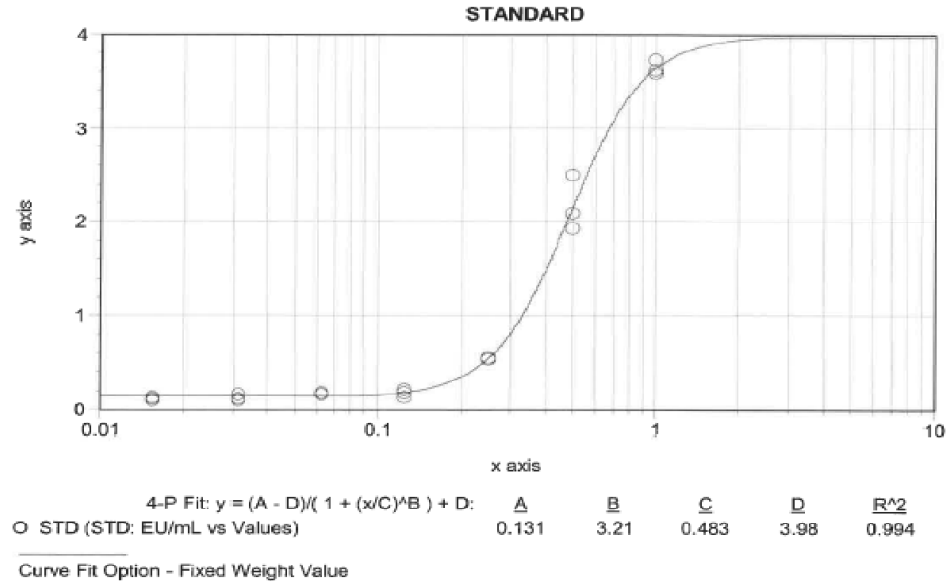

Fig. S2: IL-6 response of PBMCs to RSE standards at T3 (Y = 450nm, X = EU/mL)

Figure S3 and S4 show the WB, IL-1β T0 and T3 response to RSE ranging from 0.0313 to 2 EU/mL for both microplates, similar to that for PBMCs above. However, the curves were less steep and there was more variability between replicates. Upper and lower asymptotes were not achieved with this range of standards, but as with PBMCs, only results within range were interpolated and used for calculations.

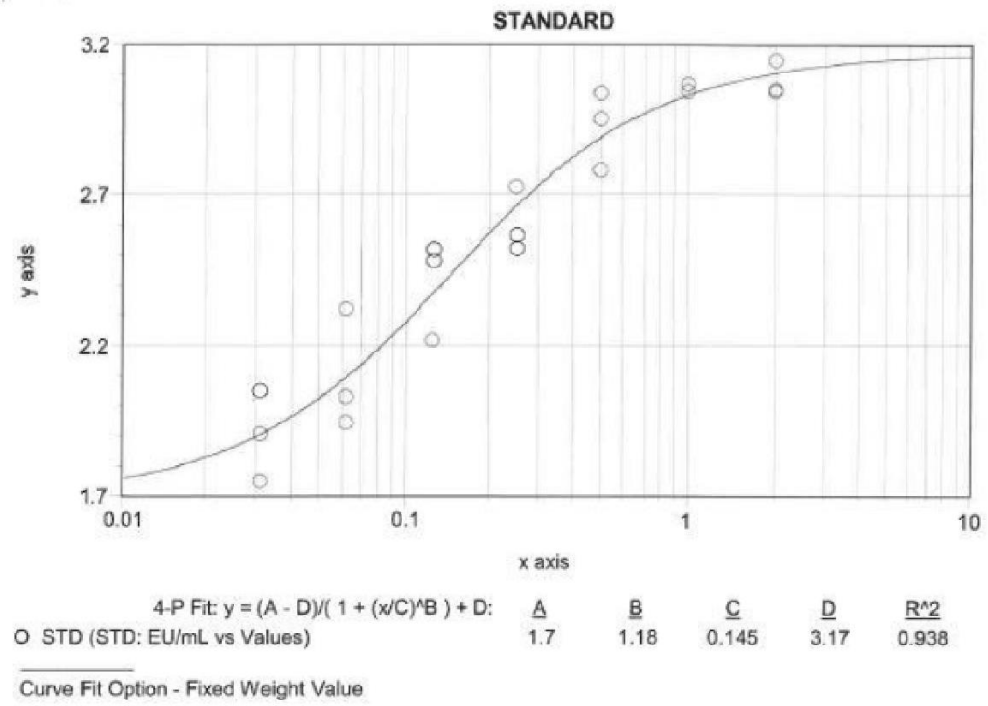

Fig. S3: IL-1β response of whole blood to RSE standards at T0 (Y = 450nm, X = EU/mL)

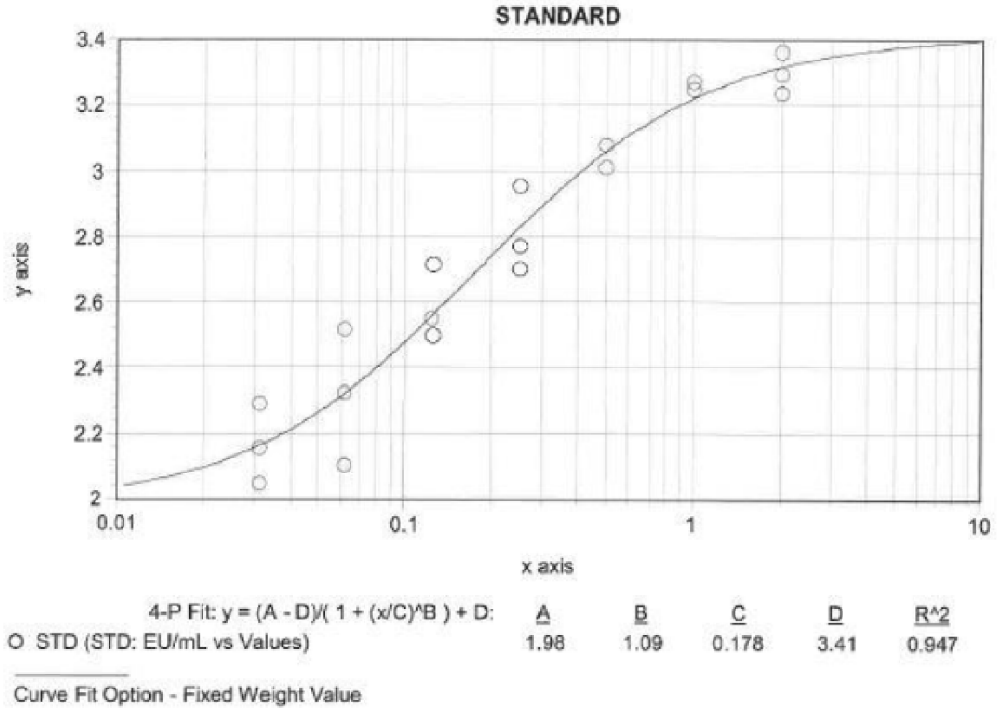

Fig S4: IL-1β response of whole blood to RSE standards at T3 (Y = 450nm, X = EU/mL)