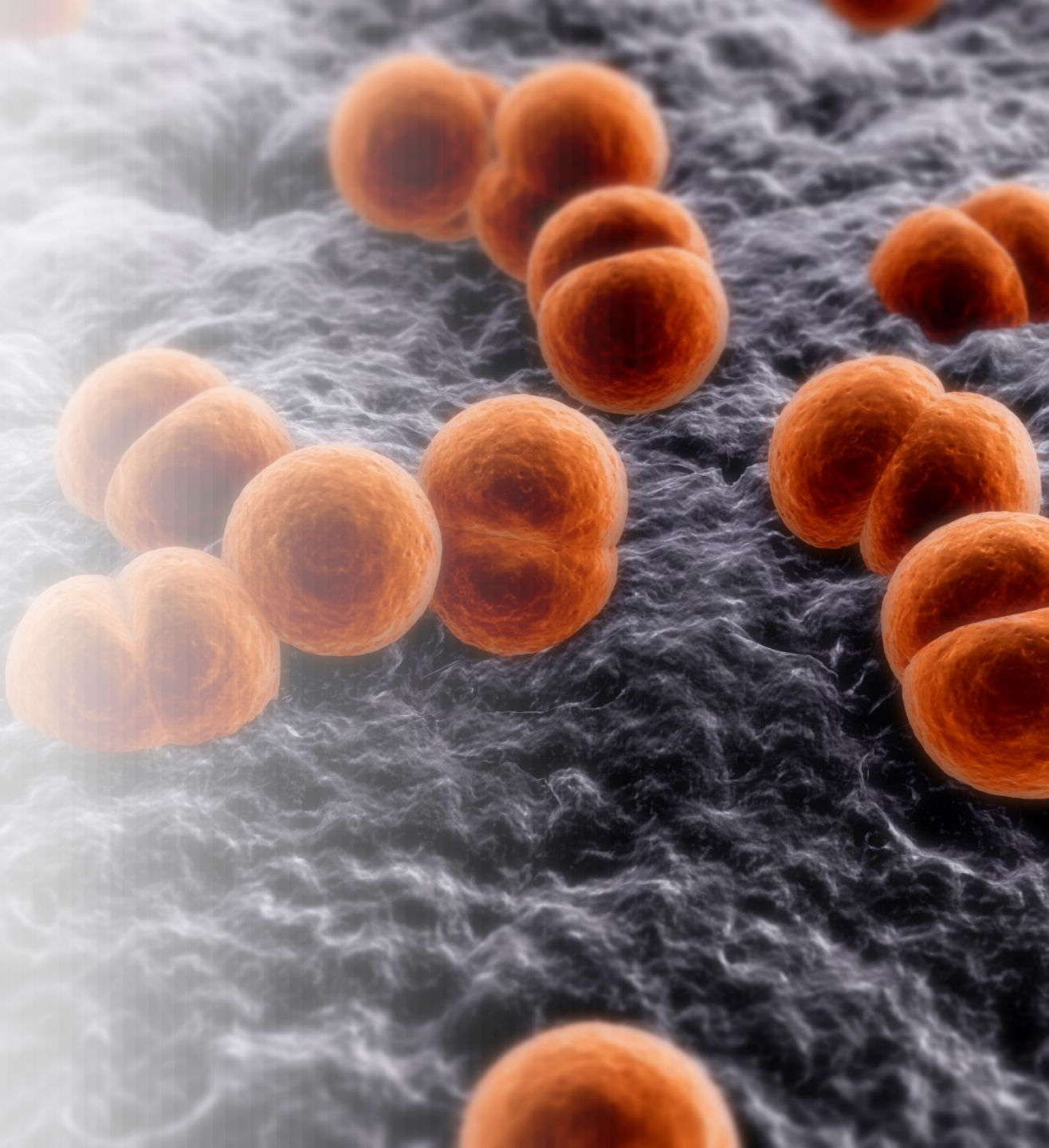
EFFECTS OF FORMULATION ON RECOVERY OF NATURAL ENDOTOXINS

Masakazu Tsuchiya, Ph. D. and Foster Jordan



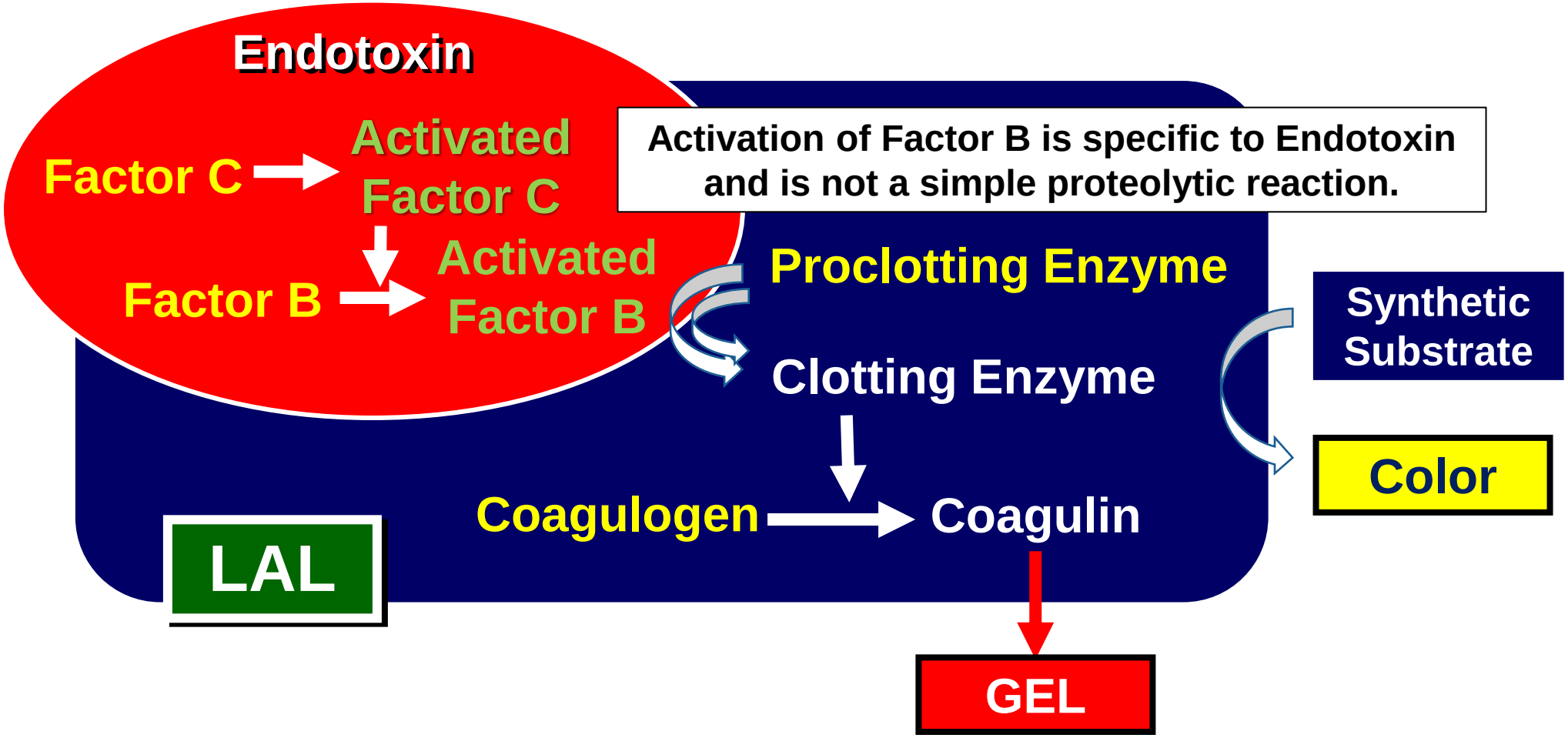
KEY LEARNINGS

Key lessons learned during the development of recombinant LAL

- A full recombinant LAL (rLAL) should be more specific to endotoxin than a recombinant FC reagent (rFC).
- LAL and rLAL have different reactivity to Reactivity of Natural Environmental Endotoxin (NEE) and Reference Standard Endotoxin (RSE).
- We should not pursue only sensitivity to Reference Standard Endotoxin (RSE).
- Optimization of rLAL formulation is necessary to avoid non detection of NEE.

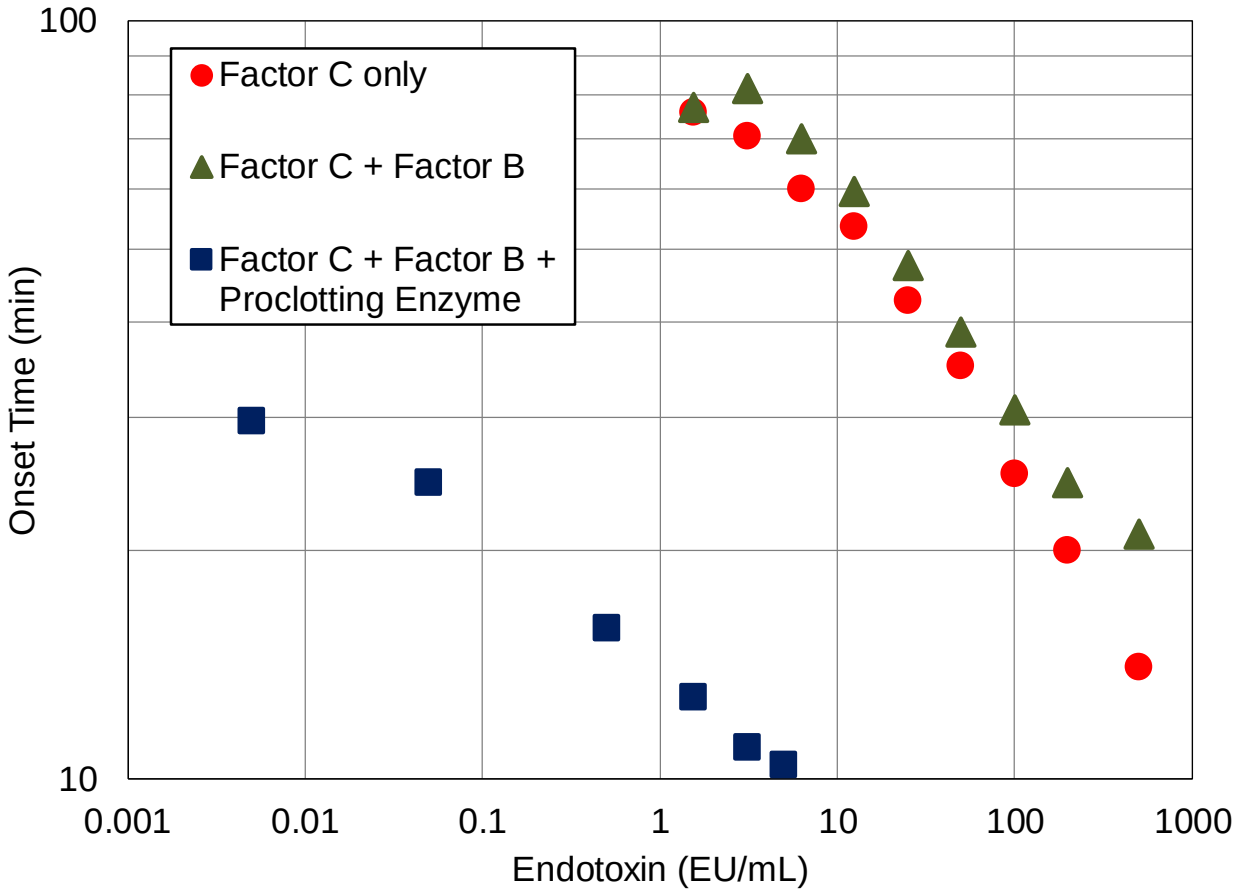
PRINCIPLE OF THE LAL TEST

Activation of Clotting Enzyme is the major driving force for amplification of the signal in the LAL.



charles river

SENSITIVITY OF RECOMBINANT REAGENTS



- Sensitivity of the reagent was dramatically increased by addition of rPCE to other factors.
- Since addition of rFB to rFC did not increase the sensitivity, activated rFC did not repeatedly activate rFB.
- These results indicates that the activation of PCE is responsible for the amplification in the LAL response.

ENDOTOXIN VALUES IN GLOBAL WATER STUDY (GWS) SAMPLES IN 2020

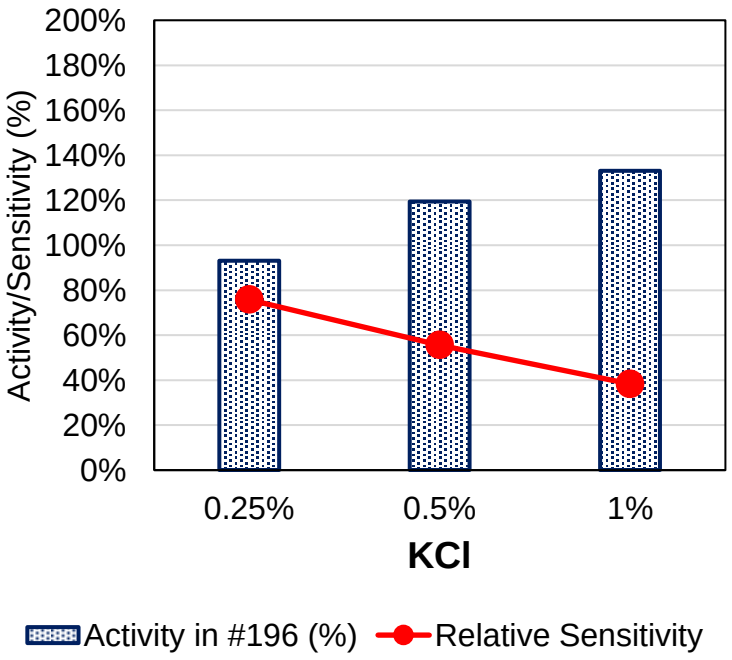
Recombinant Method	Number of Samples Tested	Number of Samples below 50% Cutoff	< 50% Cutoff Failure Rate
Pyrogene	128	100	78%
EndoZyme	128	122	95%
EndoZyme II Go	128	83	65%
rLAL	128	50	39%

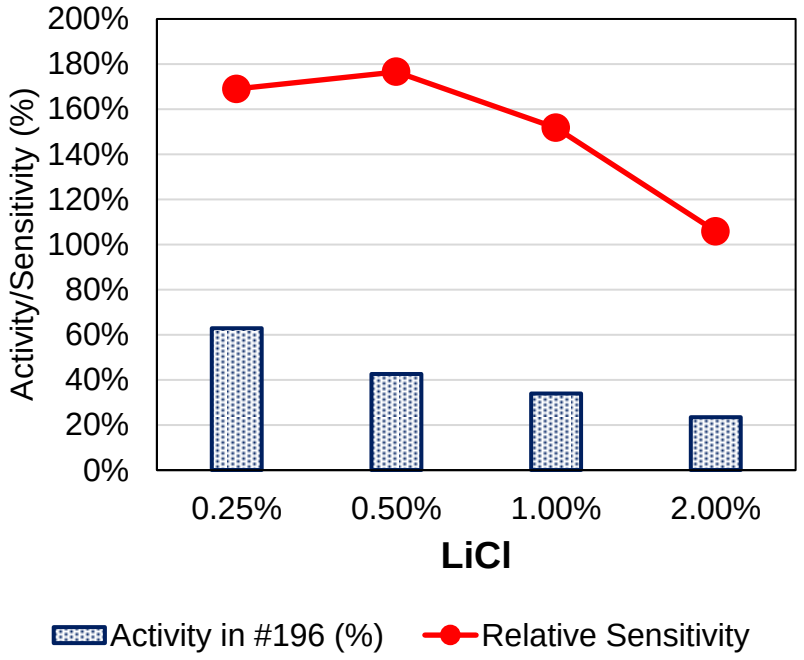
- Reactivity to NEE was not enough for the rLAL formulation.
- This should be improved because it can be a patient safety issue in endotoxin detection.

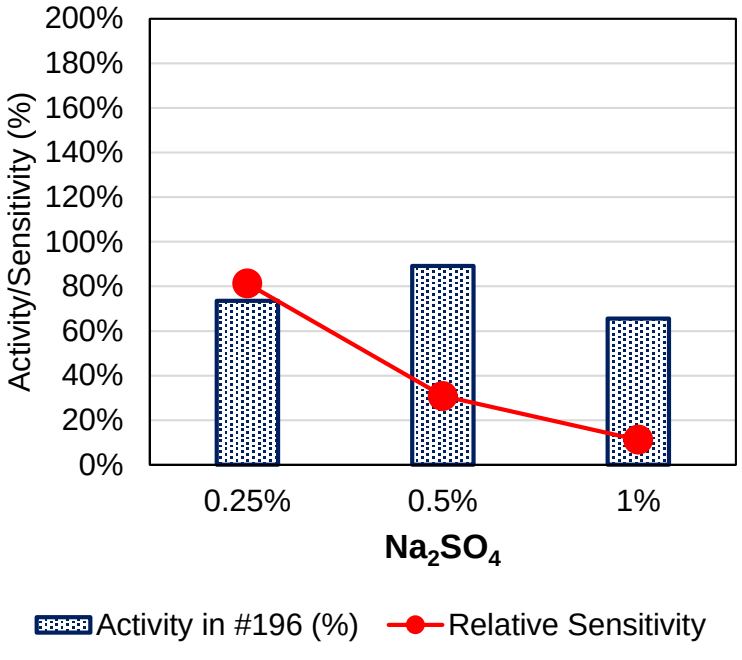
Eur J Pharm Sci, 159, (2021) 105716

EFFECT OF SALTS ON ACTIVITY OF RSE AND NEE (RLAL)

Natural Environmental Endotoxin (NEE): Water sample #196





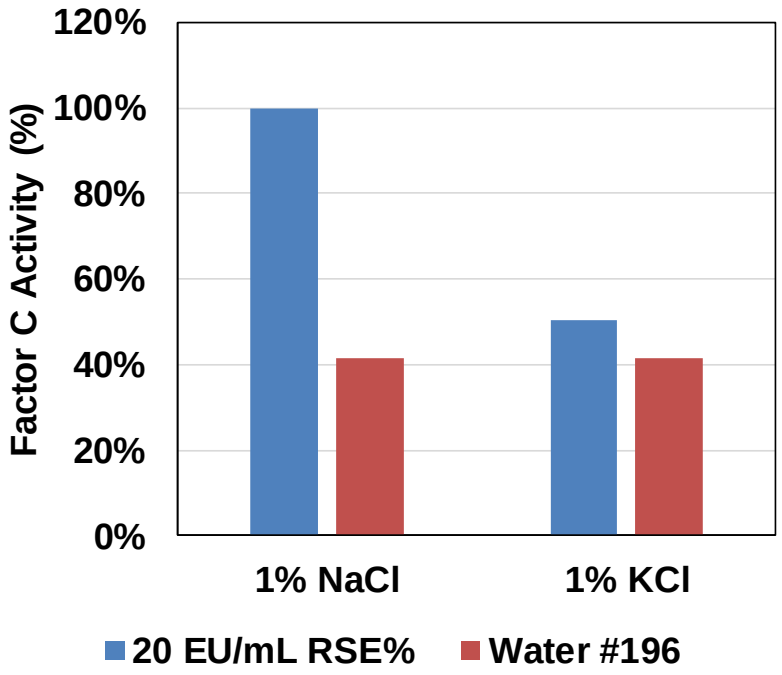


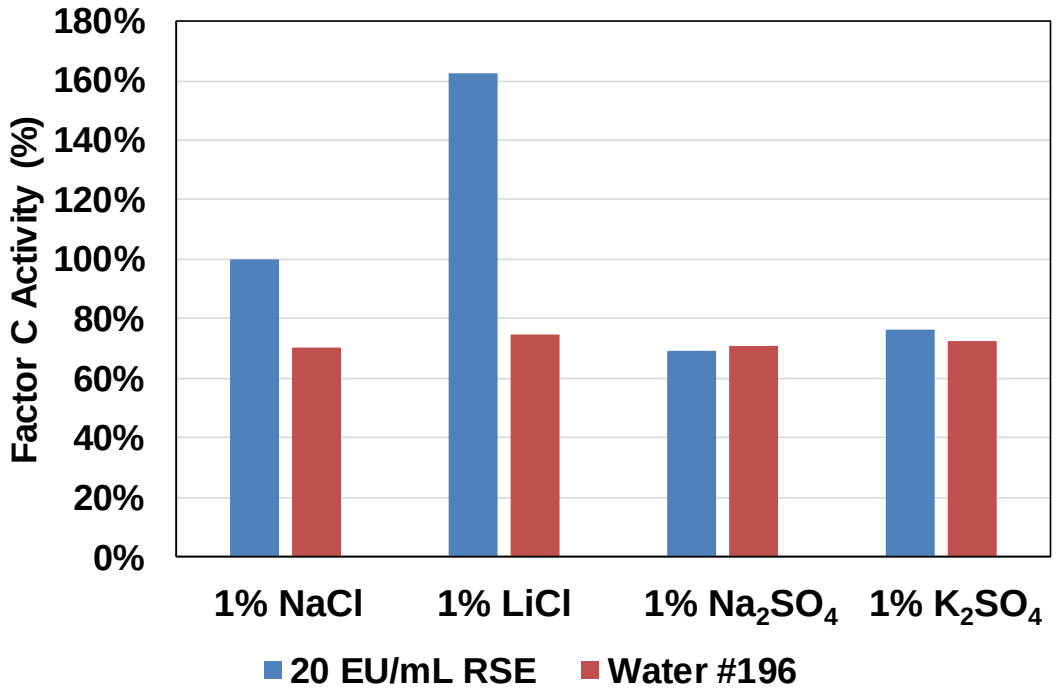
- Relative Sensitivity: Sensitivity to RSE at 0.5 EU/mL with 1.0% NaCl formulation was set at 100%.
- Relative Activity: Activity of #196 with KCA LAL was set at 100%.
- KCl inhibited RSE activity, but not NEE, resulting the calculated activity of NEE increased.
- LiCl enhanced RSE activity, but not NEE, resulting the calculated activity of NEE decreased.
- Na2SO4 inhibited RSE and NEE activity. The degree of inhibition was higher for RSE than NEE.

charles river

EFFECT OF SALTS ON RFC ACTIVATION BY RSE AND NEE

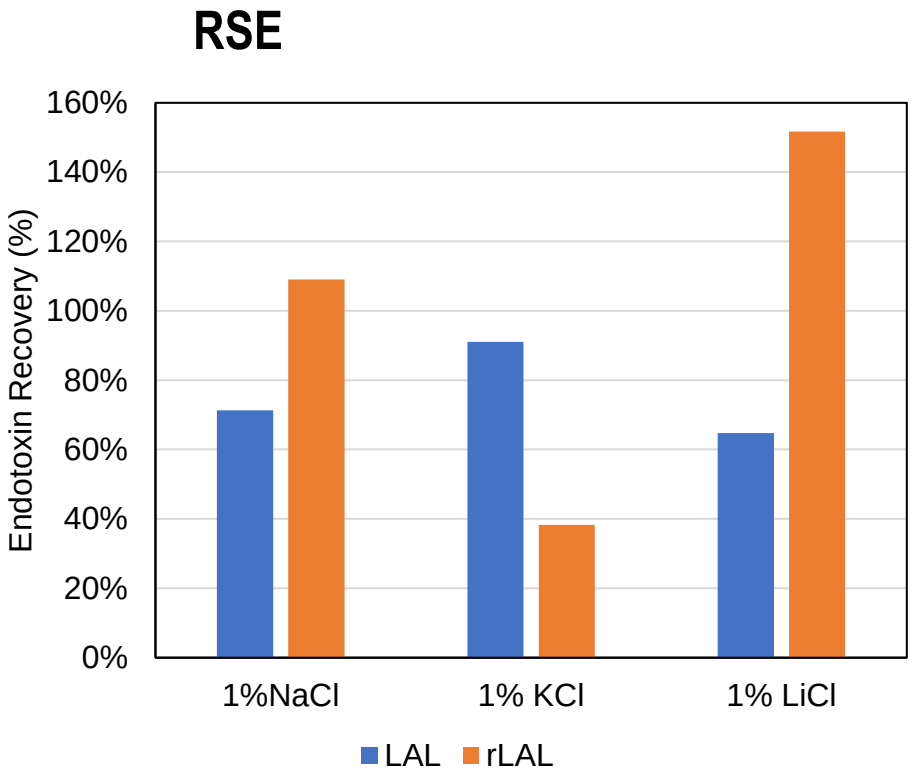
Natural Environmental Endotoxin (NEE): Water sample #196





- Factor C activation by RSE was inhibited by KCl, Na2SO4, and K2SO4, and was enhanced by LiCl.
- Factor C activation by NEE was consistent regardless of salts.
- This suggests that salts affect the rFC activation by RSE, but not by this NEE.

EFFECT OF SALTS ON LAL AND RLAL ACTIVATION BY RSE



- Effect of salts on the LAL test was not as high as rFC and rLAL.
- Salt effects on RSE in LAL activation were different from those in rLAL and rFC activation.

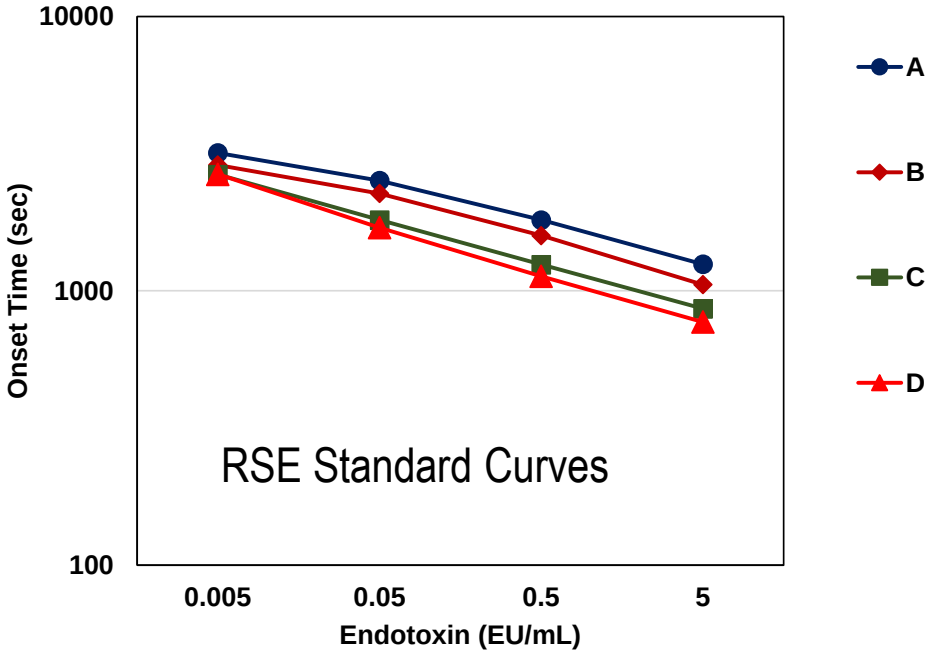
EFFECT OF DETERGENTS ON ACTIVITY OF RSE AND NEE

Natural Environmental Endotoxin (NEE): Water sample #196

Detergent	Concentration	RSE Enhancement (at 0.5 EU/mL)	NEE Activity in #196 (against KCA LAL)
A	0.05%	x 3	low
B	0.05%	x 14	low
C	0.01%	x 76	very low
D	0.002%	x 83	very low
E	0.02%	x 14	very low
F	0.006%	x 4	high
G	0.008%	x 3	low
H	0.04%	x 4	high
I	0.02%	x 5	fair
J	0.02%	x 6	fair

Type of detergent and the concentration are important for detection of NEE.

EFFECT OF FORMULATION ON RELATIVE ENDOTOXIN ACTIVITY IN WATER SAMPLES



- Each formulation contains same amounts of recombinant factors and same buffer components.
- Highest sensitivity was obtained with Formulation D.
- Formulations B, C, and D did not show enough reactivity to NEE in water samples.

Base Reagent	Formulation	Relative Endotoxin Activity (%)							
		#1	#2	#3	#4	#5	#6	#7	#8
rLAL	A	236%	222%	188%	29%	69%	647%	85%	933%
	B	0.6%	1.1%	1.4%	< 4.9%	< 2.6%	0.5%	0.4%	146%
	C	1.3%	2.7%	1.6%	< 4.9%	< 2.6%	13.8%	4.6%	99%
	D	< 0.2%	< 0.3%	< 0.3%	< 4.9%	< 2.6%	< 0.2%	< 0.1%	< 18%

WATER SAMPLES MEASURED USING DIFFERENT RLAL PREPARATIONS

Sample #	Relative Endotoxin (EU/mL)		
	rLAL for GWS	rLAL #1	rLAL #2
103	44%	151%	120%
129	40%	201%	162%
165	31%	61%	83%
196	1%	232%	170%
197	3%	128%	95%
210	38%	55%	73%
257	23%	88%	81%
305	27%	139%	67%
352	45%	175%	111%
354	23%	197%	134%
411	28%	56%	44%
613	39%	183%	165%
653	5%	217%	181%
661	10%	124%	130%
109	215%	392%	202%
135	63%	305%	242%
167	107%	201%	212%
216	122%	494%	232%
221	197%	193%	158%
Average	**56%**	**189%**	**140%**

- Improved rLAL #1 and #2 showed relatively higher activity in water samples.
- We can adjust the reactivity to NEE by using different formulations. However, we cannot set a final target for the reactivity because there is currently no NEE control.
- Our interim target is a reagent which exhibits less false negatives (underestimation), when compared to values from LAL reagents.
- Activity of KCA LAL was set at 100%.

CONCLUSION

What we Learned…

- A full recombinant LAL (rLAL) should be more specific to endotoxin than a recombinant FC reagent (rFC).
- LAL and rLAL exhibit different reactivity levels to Natural Environmental Endotoxin (NEE) and Reference Standard Endotoxin (RSE).
- We should not pursue only sensitivity to Reference Standard Endotoxin (RSE) as RSE alone is no predictor of reactivity to NEE.
- Optimization of rLAL formulation is necessary to avoid non detection of NEE.

- Need more discussions on the minimum requirements for the performance of a new recombinant reagent.

RECOMBINANT LAL DEVELOPMENT ROADMAP

Current Status

- LAL is critical patient safety test that was developed against rabbit pyrogen test with a “safety factor” that ensures patient safety.
- CRL’s recombinant LAL formulation development includes:
  - Formulations that measure the activity of both NEE's and RSE.
  - Comparing candidate recombinant formulations with LAL and USP <151> test using GMP facilities.
  - Inhibition/enhancement studies for the candidate formulations.
- Three verification lots of the best recombinant formulation.
- Establish stability and shipping.
- Enroll customer sites to compare LAL and rLAL formulation with the contaminated products at their sites.
- Evaluate results and confirm the rLAL formulation.
- Proceed to Validation studies.

Contact Us:
Masakazu Tsuchiya: Kaz.Tsuchiya@crl.com