VALIDATION OF ENDOZYME II GO - A TIME-SAVING ENDOTOXIN ASSAY BASED ON RECOMBINANT HORSESHOE CRAB FACTOR C

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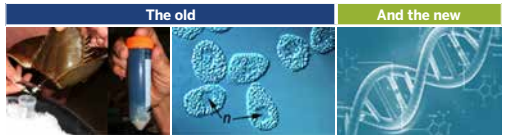
PDA Global Microbiology Meeting 2018 • Bethesda, MD

RECOMBINANT FACTOR C TESTING IN LIEU OF LAL

Given the reduction in horseshoe crab numbers as well as a drastic reduction in the numbers of shorebirds on the Atlantic seaboard, many seek to reduce pharmaceutical reliance on the Limulus test reagent (LAL). Recombinant factor C is a recombinantly produced protein expressed from the cloned horseshoe crab gene in cell culture. In this way, the reliance on the declining fix.



Blood Collection from the American Horseshoe Crab, Limulus Polyphemus, Peter Armstrong and Mara Conrad, Jour. Vis. Exp. 2008; (20): 958.

ENDOZYME® II GO - FEATURING THE GOPLATE™

The new ENDOZYME II GO is the evolution of ENDOZYME II, using the same reagents in a more efficient way. The key component, the GOPLATE, is pre-filled with CSE for the standard curve 0.005 - 50 EU/mL and PPCs 0.5 EU/mL, all in duplicate replicates (Fig. 2).

ENDOZYME II GO - fast workflow in only 3 steps:

1 - Add water and samples to the dedicated wells.
2 - Prepare and add the assay reagent.
3 - Run the assay in a fluorescence reader for e.g. 20 - 60 minutes depending on the desired sensitivity (0.05 - 0.005 EU/mL).

Fig. 1. ENDOZYME II GO kit components.

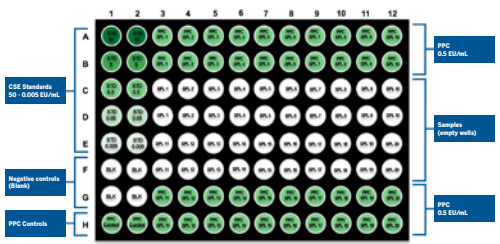
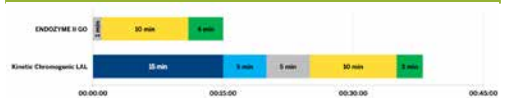


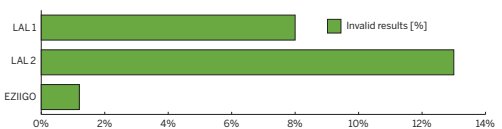
Fig. 2. Plate layout of the GOPLATE.

EASY OF USE

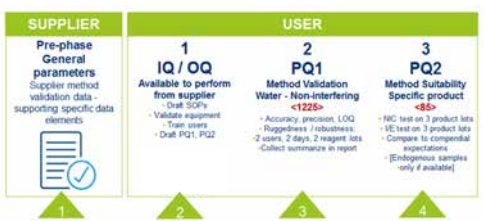
TIME SAVING



ERROR REDUCTION



ALTERNATIVE VALIDATION PER USP <1225> - LOGISTICS



INTERNAL COMPARISON STUDY

The aim of this study was to compare the new rFC test ENDOZYME II GO with kinetic-chromogenic LAL tests in terms of inclusivity, interferences, precision, exclusivity and limit of quantification. To this end, three different lots of ENDOZYME II GO were tested as well as LAL kits from two different suppliers. Fluorescence and absorbance readers used in the study included a BioTek FLx800 and a BioTek Epoch2 coupled with Gen5 software (BioTek Instruments Inc, Winooski, VT).

INCLUSIVITY

Endotoxin levels of purified lipopolysaccharide samples from 14 different strains of Gram-negative bacteria and Reference Standard Endotoxin (RSE) were tested using the ENDOZYME II GO and LAL tests from two manufacturers in parallel. The rFC test correlated well to both LAL tests with correlation coefficients of 0.948 and 0.935, respectively (Fig. 3). This correlation was slightly higher than the correlation between the two LAL tests (r = 0.933).

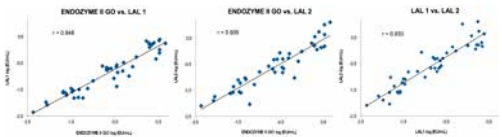


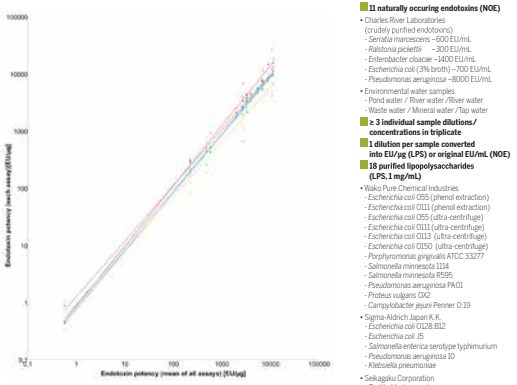
Fig. 3. Correlation of different endotoxin assays in response to 15 different LPS samples. Samples were measured in serial dilutions in single replicates (n = 44). Endotoxin values (EU/mL) were calculated from the respective standard curve and are plotted as logarithm. R values were calculated by Pearson correlation tests. A.) ENDOZYME GO vs. LAL1 B.) ENDOZYME II GO vs. LAL2 C.) LAL1 vs. LAL2

RFC to LAL is closer in correlation than LAL1 to LAL2.

KIKUCHI ET AL. 2017. COLLABORATIVE STUDY

on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides. Pharmaceutical and Medical Device Regulatory Science 48(4), 252–260. [Japanese]

no.	naturally occurring endotoxin 由来	測定値(EU/mL)** Endospecy	ES-II	Kinetic-QCL	PyroSmart	PyroGene	Endozyme
	native endotoxin						
1	*Escherichia coli*	543	621	554	404	818	743
2	*Enterobacter cloacae*	897	1329	1176	298	1287	1098
3	*Pseudomonas aeruginosa*	2400	4141	2768	2840	3376	2456
4	*Ralstonia pickettii*	214	360	254	92	454	244
5	*Serratia marcescens*	400	504	447	108	459	312
	水						
6	蒸留水	95.6	100.7	139.5	62.7	72.0	35.3
7	阿川水 1	222.0	247.6	295.0	244.5	231.0	134.0
8	阿川水 2	204.5	284.4	303.5	82.5	198.5	98.0
9	生活排水（家庭排水用浄化槽）	111.0	160.3	164.0	86.0	138.0	77.9
10	市販ミネラルウォーター	0.114	0.116	0.140	0.088	0.084	0.080
11	水道水	8.105	10.964	14.820	10.285	4.830	1.295



INTERFERENCE TESTING

For evaluation of interferences, different samples such as common excipients, proteins, organic solvent and culture medium were tested in duplicates without and with PPCs of 0.5 EU/mL. A PPC recovery of 50-200% indicated a valid result. All three methods were comparable regarding tolerated concentrations of substances for valid PPCs. The rFC test yielded a higher rate of valid recoveries (Tab. 1.)

Category	Substance	Conc./ dilution	PPC recovery in ENDOZYME® II GO [%]	PPC recovery in LAL1 [%]	PPC recovery in LAL2 [%]
Common excipients	Sodium citrate	1 mM	112	161	92
		0.1 mM	106	161	118
	Dextrose	5%	80	134	92
		0.5%	95	165	108
	NaCl	500 mM	58	57	**1**
		50 mM	92	135	84
	Polysorbate 20	0.02%	77	61	50
		0.002%	101	69	**43**
	PBS	1x	82	96	**36**
		1:10	100	142	110
Proteins	MAB-33	0.1 mg/mL	117	**603**	**46**
		0.01 mg/mL	108	190	92
	HSA	1 mg/mL	94	**332**	138
		0.1 mg/mL	111	188	76
Organic solvent	Ethanol	1%	99	135	94
		0.1%	114	174	104
Culture medium	IMDM	100%	**35**	**214**	55
		10%	91	**262**	130

Tab. 1. Percentage of PPC recovery (0.5 EU/mL) in different samples in ENDOZYME® II GO compared to two LAL assays. Samples and spiked samples were tested in duplicate replicates (n = 2). Red font color highlights invalid PPC recovery.