

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

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## 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 (n=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.

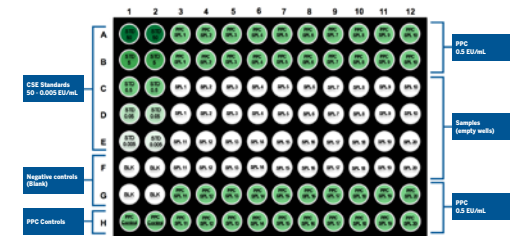
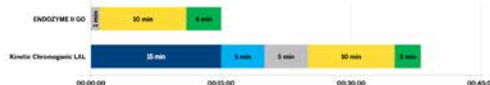


Fig. 2. Plate layout of the GOPLATE.

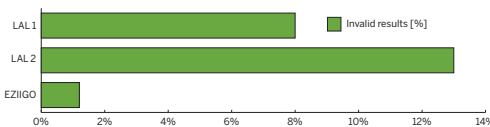
## EASY OF USE



## TIME SAVING



## ERROR REDUCTION



## ALTERNATIVE VALIDATION PER USP <1225> - LOGISTICS

SUPPLIER	USER
<p><b>Pre-phase General parameters</b></p> <ul style="list-style-type: none"> <li>Supplier method validation data supporting specific data elements</li> </ul>	<p><b>1 IQ/OQ</b></p> <p>Available to perform from supplier</p> <ul style="list-style-type: none"> <li>Draft SOPs</li> <li>Validate equipment</li> <li>Train users</li> <li>Draft PQ1, PQ2</li> </ul>
	<p><b>2 PQ1</b></p> <p>Method Validation Water - Non-interfering &lt;1225&gt;</p> <ul style="list-style-type: none"> <li>Accuracy, precision, LOQ</li> <li>Robustness / robustness</li> <li>2 users, 2 days, 2 reagent lots</li> <li>Collect summary in report</li> </ul>
	<p><b>3 PQ2</b></p> <p>Method Suitability Specific product &lt;85&gt;</p> <ul style="list-style-type: none"> <li>HC test on 3 product lots</li> <li>Endogenous samples - only if available</li> <li>Vegetations</li> </ul>

## 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).

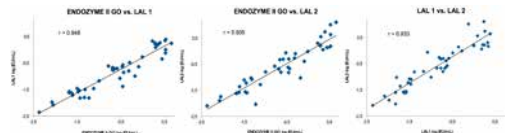


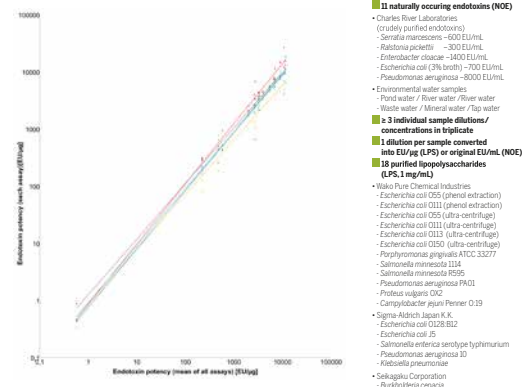
Fig. 3. Correlation of different endotoxin assays in response to 15 different LPS samples. Samples were measured in serial dilutions in single replicates (n=4). Endotoxin values (EU/mL) were calculated from the respective standard curve and are plotted as logarithm. R values were calculated by Pearson correlation tests. A) ENDOZYME II 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. 品名	Endoscopy	IS-II	測定値(EU/mL)*			
			Kinetic-QCL	PyroSmart	PyroCera	EndoZyme
1 天然産毒素						
1 <i>Escherichia coli</i>	543	401	554	404	818	743
2 <i>Acinetobacter baumannii</i>	897	1329	1176	290	1287	1098
3 <i>Pseudomonas aeruginosa</i>	3400	4141	2768	2849	3376	3464
4 <i>Escherichia coli</i> O157	214	303	254	92	484	244
5 <i>Salmonella enterica</i>	400	504	447	108	459	312
6 水						
7 精製水	95.6	100.7	139.5	42.7	75.0	85.8
8 河川水 1	222.0	247.6	259.5	244.5	231.0	134.0
8 河川水 2	204.5	254.4	303.5	82.5	198.6	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	77.0
10 高級ろ過水 (浄化槽水用浄化槽)	0.114	0.116	0.140	0.088	0.054	0.080
11 水道水	8.105	10.964	14.820	10.285	4.820	1.295



**11 naturally occurring endotoxins (NOE)**

- Charles River Laboratories (crude purified endotoxins)
- Seratiol macresens - 600 EU/mL
- Rabdonia pacifica - 300 EU/mL
- Enterobacter cloacae - 1400 EU/mL
- Escherichia coli (3% broth) - 700 EU/mL
- Pseudomonas aeruginosa - 8000 EU/mL

**Environmental water samples**

- Hand water / River water / River water
- Waste water / Mineral water / Tap water

**2 3 individual sample dilutions/concentrations in triplicate**

**11 dilution per sample converted into EU/µg (LPS) or original EU/mL (NOE)**

**15 purified lipopolysaccharides (LPS, µg/mL)**

- Wako Pure Chemical Industries
- Escherichia coli O26 (general extraction)
- Escherichia coli O111 (general extraction)
- Escherichia coli O25 (ultra-centrifuge)
- Escherichia coli O111 (ultra-centrifuge)
- Escherichia coli O25 (ultra-centrifuge)
- Escherichia coli O250 (ultra-centrifuge)
- Pseudomonas glauca ATCC 32777
- Salmonella mitsubashi 114
- Salmonella mitsubashi R555
- Pseudomonas aeruginosa PAD1
- Proteus vulgaris O2G
- Campylobacter jejuni bacter 019
- Sigma-Aldrich Japan K.K.
- Escherichia coli O128 B12
- Escherichia coli S.
- Salmonella enterica serotype typhimurium
- Pseudomonas aeruginosa 30
- Klebsiella pneumoniae
- Sekisui Corporation
- Burkholderia cepacia

## 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 [%]	
			(n)	(%)	(n)	(%)	(n)	(%)
Common excipients	Sodium citrate	1 mM	112	161	161	118		
		0.1 mM	106	161	134	92		
		5%	80	134	134	108		
	Dextrose	0.5%	95	165	108	108		
		500 mM	98	97	1	1		
		0.002%	101	69	43	46		
Polysorbate 20	1x	112	96	36	36			
	1:10	100	142	110	110			
	1:100	107	63	46	46			
Proteins	MAB 33	0.1 mg/mL	117	190	190	92		
		0.01 mg/mL	108	190	138	138		
		1 mg/mL	94	332	332	138		
HSA	0.1 mg/mL	111	188	76	76			
	1%	99	135	94	94			
	0.1%	114	174	104	104			
Culture medium	IMDM	100%	35	214	214	55		
		10%	91	120	120	120		

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.