Comparison of 3 Different Endotoxin Detection and Quantification Methods

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PURPOSE: To perform and compare chromogenic, turbidimetric, and gel clot methods of endotoxin detection based on the efficacy, sensitivity and cost effectiveness of each test.

INTRODUCTION: Endotoxins are structural components of gram-negative bacteria known to cause toxicity and contamination in laboratory samples. The most common source of endotoxin is lipopolysaccharide, found in the outer membrane of the bacterial cell wall. It is known that the lipid portion of lipopolysaccharide contributes to toxicity, capable of inducing a strong response from normal animal immune systems.

HISTORY: Chromogenic, turbidimetric, and gel clot tests make use of the Limulus Amoebocyte Lysate (LAL). It was serendipitously discovered from the observation that a Gram negative infection of *Limulus polyphemus,* the horseshoe crab, resulted in fatal intravascular coagulation. Observed coagulation occurred as a reaction between endotoxin and amoebocytes circulating in Limulus blood. The clottable protein from Limulus Amoebocytes was extracted and is used as a sensitive indicator of endotoxin presence.

METHODOLOGY: Two protein samples were obtained and tested for endotoxin contamination using chromogenic, turbidimetric, and gel clot testing kits purchased from LONZA. All materials used for testing are endotoxin-free. All reagents were adjusted to pH 6 – 8 range.

1. QCL-1000® Chromogenic LAL Endpoint Assay

The chromogenic test is a colourimetric, quantitiative assay that detects the level of endotoxin in a sample by the measurement of its absorbance value. The activation of LAL by endotoxin catalyses the cleavage of a colourless substrate, releasing the chrompophore pNA. The intensity of yellow colour given off by pNA is an indirect indication of endotoxin concentration in the sample. The concentration of endotoxin can be calculated using a standard curve.

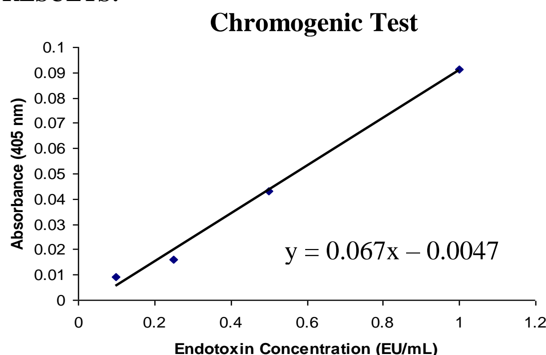
2. LAL PYROGENT®-5000

The turbidimetric test is a kinetic, quantitative assay for the detection of endotoxin. Activated LAL converts coagulogen to coagulin to form turbidity in the sample. The increase in optical density is measured as reaction time and is inversely correlated with the level of endotoxin found in the sample. The concentration of endotoxin can be calculated using a standard curve.

3. PYROGENT® Plus Single Test Kit LONZA

The gel clot test is a semi-quantitative test of endotoxin detection. Each vial contains LAL freeze-dried in powdered form. Injection of sample in the vial activates the reaction. Clotting of sample can be determined by turning the vial upside-down after the incubation period.

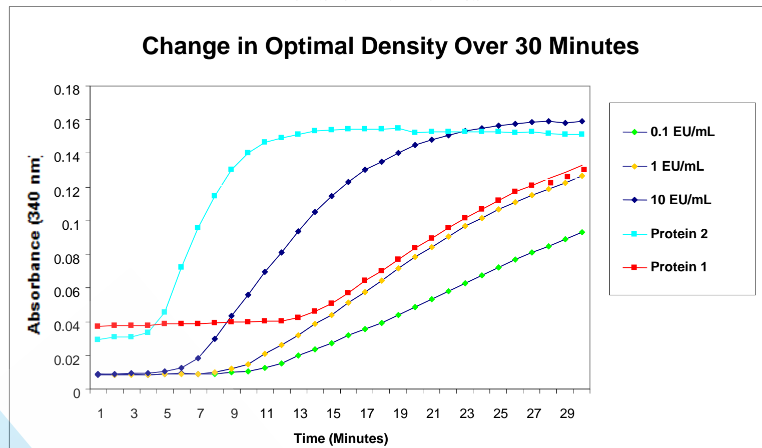
RESULTS:



Protein 1:
0.51 EU/mL

Protein 2:
1.034 EU/mL

Turbidimetric Test



Protein 1 had a reaction time of 19 minutes; Protein 2 had a reaction time of 9 minutes. Protein 1 contains ~ 1 EU/mL and Protein 2 contains ~ 10 EU/mL.

Gel Clot Test (duplicate assay)

0.5 EU/mL	0.25 EU/mL	0.125 EU/mL	0.0625 EU/mL	0.03125 EU/mL	NC	Protein 1	Protein 2
+	+	+	+	–	–	–	+
+	+	+	+	–	–	–	+

Protein 1 contains less than 0.0625 EU/ml endotoxin.
Protein 2 contains more than 0.0625 EU/mL endotoxin.
*+ indicates clot formation

Summary

METHOD	DETECTION LIMIT	LENGTH OF TEST	# of TESTS/KIT	KIT PRICE	COST/TEST	SAMPLE VOLUME
QCL-1000® Chromogenic LAL Endpoint Assay 50-647U	0.1 EU/mL	1 hr	120	$447.00	$ 3.725	50 µl
PYROGENT®-5000 Kinetic Turbidimetric Assay N383	0.01 EU/mL	1.5 hr	20 - 100	$184.00	$1.84 – $ 9.2	100 µl
PYROGENT® Plus Single Test Kit N289-125	0.125 EU/mL	0.5 hr	4 - 11	$105.00	$ 9.55 - $ 26.25	250 µl

CONCLUSION: Chromogenic, turbidimetric, and gel clot tests are effective methods of endotoxin detection. The three different tests are quick and easy to use with the gel clot test being the fastest but most expensive (and least quantitative). Qualitatively all three were consistent in indicating the same protein sample of the two tested had the higher endotoxin level, although the different methods produced significantly different results for the same sample.