

ENDOZYME® II GO AUTOMATED ENDOTOXIN DETECTION ASSAY PROTOCOL USING ANDREW+



Your Ally in Advancing Quality

Introduction/Background

Use of the Andrew+ Robot and OneLab to Automate Liquid Handling for Bacterial Endotoxin Testing using ENDOZYME® II GO

Bacterial Endotoxin Testing (BET) is a mandatory quality control step in the release of injectable pharmaceuticals, biologics, and medical devices. Endotoxins are lipopolysaccharides (LPS) found in the outer membrane of Gram-negative bacteria. If they enter a patient's bloodstream even at very low concentrations via contaminated parenteral medications or medical devices, they can trigger severe immune reactions which could lead to death.

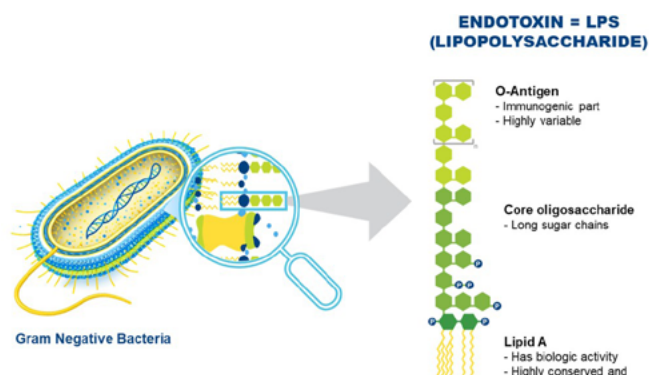


Figure 1: Bacterial Endotoxins are released by the membrane of Gram-negative bacteria

For over 40 years, the test for bacterial endotoxins has been performed by conventional Limulus Amoebocyte Lysate (LAL) which rely on the blood of the horseshoe crab (*Limulus polyphemus*), an ancient marine animal whose hemolymph contains a cascade of serine proteases that react specifically to bacterial endotoxins. The dependence on a wild-harvested animal raises concerns around supply security, lot-to-lot variability, and ethical acceptability in the context of the 3Rs principles (Replace, Reduce, Refine).

Exemplary Data for ENDOZYME® II GO generated with this Andrew+ protocol

For each linear standard curve range (5 – 0.05; 20 min incubation time & 5 – 0.005 EU/mL; 60 min incubation time) 10 GOPLATES were prepared by the Andrew+ liquid handling system and measured afterwards with a Synergy HTX reader. In the following figures the distribution of the standard curve parameters is shown.

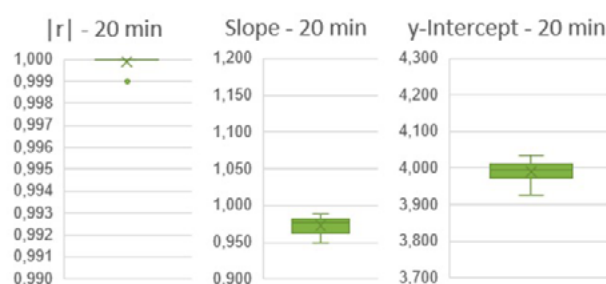


Figure 2: Distribution of standard curve parameter for the 3-point linear standard curve (5 - 0.05 EU/mL) measured with 20 min total incubation time. $n = 10$



Figure 3: Distribution of standard curve parameter for the 4-point linear standard curve (5 - 0.005 EU/mL) measured with 60 min total incubation time. $n = 10$

All 10 plates were filled with endotoxin-free water as samples. To evaluate the accuracy of the setup, 210 samples were evaluated. In the following figures the distribution of the acquired Positive Product Control (PPC) recoveries is shown:

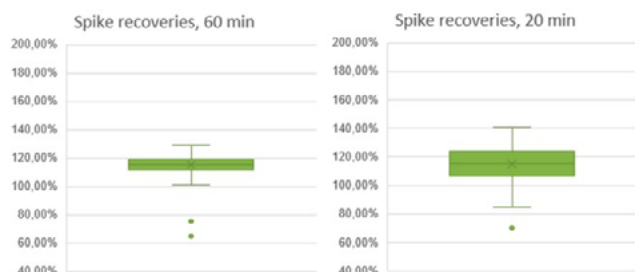
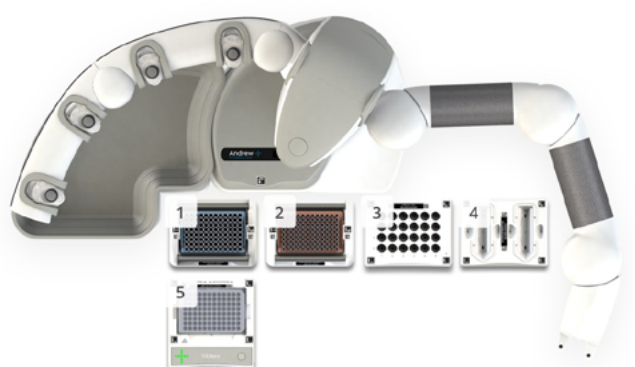


Figure 4: Spike recovery distribution of 210 samples measured on 10 plates for each standard curve range. Left: 5 - 0.005 EU/mL with 60 min incubation time. Right: 5 - 0.05 EU/mL with 20 min incubation time.

Assay notes

As the instructions indicate, all materials used for assay execution must be free of detectable endotoxins. A clean environment is strongly recommended to execute these assays.



Position	Dominos and connected devices
1-2	Tip Insertion System Domino
3	16mm Vial Domino
4	8-Channel Reservoir Domino
5	Microplate Peltier+



Figure 5: Andrew+ OneLab deck set up for endotoxin testing with ENDOZYME[®] II GO kit. Domino/Device locations on Andrew+ deck: [1-2] Tips Insertion System Domino, [3] 16mm Vial Domino, [4] 8-Channel Reservoir Domino, [5] Microplate Peltier+

Protocol specifications

ENDOZYME[®] II GO 0.005 EU/mL:

- Standard curve range:
 - 5 – 0.005 EU/mL (4-points, 60 min)
 - 5 – 0.05 EU/mL (3-points, 20 min)
- Estimated time of execution: 21m
- Hands-on time: 5m
- Tip consumption:
 - 8 x 10-300 µL tips
 - 22 x 50-1000 µL tips
- Max samples per plate: 21

ENDOZYME[®] II GO 0.01 EU/mL:

- Standard curve range: 1 – 0.01 EU/mL (3-points, 60 min)
- Estimated time of execution: 22m
- Hands-on time: 5m
- Tip consumption:
 - 8 x 10-300 µL tips
 - 23 x 50-1000 µL tips
- Max samples per plate: 22

Protocols²

ENDOZYME[®] II GO 0.01 EU/mL

- Testing 22 samples with no dilution (or previously diluted) with a 3 points standard curve (1 to 0.01 EU/mL)

ENDOZYME[®] II GO 0.005 EU/mL

- Testing 21 samples with no dilution (or previously diluted) with a 4 points standard curve (5 to 0.005 EU/mL)

Materials and Consumables

Andrew+ System Components: Dominos, Devices, Electronic Pipettes & Tips

- Andrew+ Pipetting Robot
- OneLab software
- 2× Tip Insertion System Domino | p/n 186009612
- 8 Channel Pipette reservoir | p/n 186009613
- Ø16 mm Vial Domino | p/n 186010308
- Microplate Peltier+ | p/n 176004852
- Andrew Alliance Bluetooth Electronic Pipette, 1-ch 1000 µL | p/n 186009766
- Andrew Alliance Bluetooth Electronic Pipette, 8-ch 300 µL | p/n 186009607

Recommended consumables

- bioMérieux ENDOZYME® II GO Bulk | p/n 423427
- bioMérieux ENDOZYME® II GO 0.01 Bulk | p/n 423533
- bioMérieux EndoGrade Glass Test Tubes | p/n 800050
- Sartorius Optifit Racked Pipette Tips 50-1200 µL | p/n 791200
- Sartorius Optifit Racked Pipette Tips 5-350 µL | p/n 790350
- INTEGRA 10 mL multichannel reservoir | p/n 4332

References

1. ENDOZYME® II GO Bulk brochure (<https://www.biomerieux.com/content/dam/biomerieux-dam/Industry/products-and-services/pharma-quality-control/endotoxins/endozyme%C2%AE-2-go/PHARMA-BROCHURE-EZIIGO-A4-05-23-9324100-Pages.pdf.coredownload.pdf>)
2. ENDOZYME® II GO 0.01 Brochure (https://www.biomerieux.com/content/dam/biomerieux-dam/Industry/products-and-services/pharma-quality-control/endotoxins/endozyme%C2%AE-2-go/PHARMA_BROCHURE-ENDOZYME-II-GO-0-01-PLATE-9329998_Pages.pdf.coredownload.pdf)

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