FASTER, FURTHER - RELIABLY AND SUSTAINABLY: A NEW PRODUCT OFFERING FOR RECOMBINANT FACTOR C ENDOTOXIN TESTING

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BACKGROUND

The vast majority of endotoxin tests for pharmaceuticals and medical devices as well as their in-process intermediates and raw materials are microplate-based. They require tedious reconstitution and dilution steps for preparation of Control Standard Endotoxin (CSE) dilutions and Positive Product Controls (PPCs). These time-consuming manual handling steps can result in substantial variability and a significant rate of invalid results that demand repeat testing.

To address these issues as well as to reduce the time for microplate preparation, we have developed a ready-to-use microplate - the GOPLATE™ - embedding required CSE amounts in dried format. Thus, the conventional standard dilution has become completely obsolete, in turn the GOPLATE significantly lowers the risk for human error and cost-intensive test repetition. The GOPLATE is included in a complete test kit, **ENDOZYME® II GO.**

The new microplate is the first of its kind, ready-to-use and enables more than 50% reduction in handling-time as well as consistent standard curve and PPC accuracy.

RECOMBINANT HORSESHOE CRAB FACTOR C (RFC)

Recombinant Horseshoe Crab Factor C (rFC) is an exact synthetic copy of the endotoxin-sensitive enzyme naturally harboured by the blood of horseshoe crabs. Compared with Limulus amebocyte lysate (LAL) methods, endotoxin detection assays based on rFC offer:

Specificity, flexibility, lot-to-lot consistency and, importantly: a sustainable, animal-saving and secure source.

rFC tests are available as fluorescence end-point assays in 96-well microplate format and validated in exactly the same way as conventional methods according to pharmacopoeial Bacterial Endotoxin Testing criteria. The US FDA included rFC assays in the Guidance for Industry in 2012 and, in 2016, the European Pharmacopoeia followed suit. Recently, the Japanese Pharmaceutical and Medical Device Agency published a collaborative study demonstrating equivalence between rFC and LAL assays.

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.

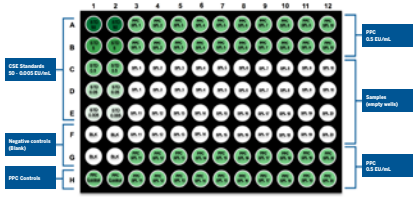


Fig. 2. Plate layout of the GOPLATE.

AIM OF STUDY & MATERIALS

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).

RESULTS

INCLUSIVITY

The accuracy was evaluated using three lots and a different microplate per lot with two different ways: accuracy of CSE standard curve compared to back-calculated concentrations (BCCs) and accuracy of CSE standard curve compared to RSE standard curve. The BCCs were close to 100% in all cases and fulfilled the criteria.

CSE / BCC	Mean	Max	Min
Lot 1 (n = 8)	100%	104%	94%
Lot 2 (n = 6)	100%	107%	91%
Lot 3 (n = 6)	100%	105%	93%

CSE / RSE	Mean	Max	Min
Lot 1 (n = 8)	90%	108%	80%
Lot 2 (n = 6)	92%	106%	84%
Lot 3 (n = 6)	117%	129%	108%

Fig 3: Accuracy of the ENDOZYME II GO on CSE or RSE. Acceptance criteria for CSE/BCC is 75-133% of the nominal concentration and for the CSE/RSE comparison, acceptance criteria is the mean of BCCs have to have an accuracy of 75 – 133% between RSE and CSE concentration.

INTER ASSAY PRECISION

The inter assay precision was evaluated on three lots with pre-loaded CSE, RSE and one endotoxin quality control sample at three different levels: high, medium and low concentrations in triplicates were tested in three different runs.

Lot 1	Mean CV	Max CV	Min CV
Run 1	3%	7%	1%
Run 2	3%	8%	0%
Run 3	3%	7%	0%

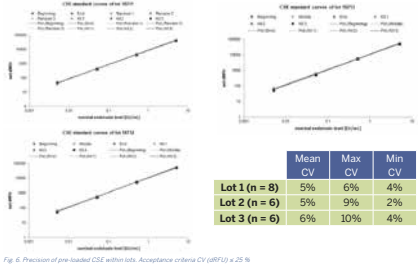
Lot 2	Mean CV	Max CV	Min CV
Run 1	5%	9%	2%
Run 2	4%	10%	1%
Run 3	4%	10%	1%

Lot 3	Mean CV	Max CV	Min CV
Run 1	3%	11%	0%
Run 2	4%	14%	1%
Run 3	3%	8%	0%

Fig. 4. Precision inter assay. Acceptance criteria CV (dRFU) ≤ 25%

CSE STANDARD CURVE

The pre-filled standard curve was tested on three different lots evaluating the CVs for the different concentrations. CSE standard curves were fitted to the CSE net increase in fluorescence intensity [net dRFU] vs. the nominal endotoxin level [EU/mL].



	Mean CV	Max CV	Min CV
Lot 1 (n = 8)	5%	6%	4%
Lot 2 (n = 6)	5%	9%	2%
Lot 3 (n = 6)	6%	10%	4%

Fig. 6. Precision of pre-loaded CSE within lots. Acceptance criteria CV (dRFU) ≤ 25 %

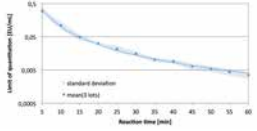
PREPARATION TIME

An experiment was performed in a routine lab to quantify the plate preparation time for the ENDOZYME II GO and a Kinetic Chromogenic method. The overall time and the time to perform each step were determined. Due to the embedded standard curve and PPCs some steps are eliminated and the preparation time of a GOPLATE was shown to be 15 minutes. As comparison, the time required to manually prepare a microplate for kinetic chromogenic LAL was 38 minutes.

Fig 7. Clocking study performed in a routine lab comparing the traditional method to ENDOZYME II GO for the different step of the microplate preparation.

LIMIT OF QUANTIFICATION

The limit of quantification was evaluated using three different reagent lots of ENDOZYME II GO and correlated to time to result. After 15 minutes, 0.05 EU/mL were detected and within 55 minutes 0.005 EU/m.



CONCLUSION

The new method based on rFC - ENDOZYME II GO - has been validated according to compendia chapters fulfilling all required criteria.

Implementation of an rFC method requires a limited effort and investment:

- To **reduce the risk of supply** (impact on product release)
- To use a **sustainable source** of reagent for a **long term vision**
- To get a **lot to lot consistency** with a better standardization
- To bring a **higher flexibility** for the lab organization: easy to handle and time saving.

From a workflow point of view, our new method requires **less than half of the handling time** in comparison to conventional microplate-based endotoxin tests.

The GOPLATE **helps reduce the risk of human error** and invalid results by eliminating the manual preparation of standard dilutions and PPCs.

Hands-on time reduction combined with shorter time to result, e.g. for water testing, provides **a high-throughput solution.** Furthermore, the simplified workflow facilitates automation.