The CMD αBET™ endotoxin testing system

Overview of the system

The CMD αBET™ system is a fully integrated testing system that provides a rapid and sustainable solution to endotoxin testing without compromising on sensitivity or performance. Using a fraction of FDA-licensed turbidimetric Limulus amoebocyte lysate (LAL) reagent, quantitative results are provided in under half the time of other testing formats, with minimal user input. The on-board FDA part 11 ready software walks users through a pharmacopoeial compliant assay whilst ensuring data integrity is maintained. The αBET™ system is designed for in-process or final-product testing in pharmaceutical manufacturing and quality control as well as in research and development.

Like other turbidimetric assays, αBET® measures changes in the transmission of light passing through a sample to monitor the progress of the reaction. The instrument employs a 4-channel consumable to enable four simultaneous tests with run times of between ~3 minutes for a 10 EU/mL sample and ~30 minutes for samples containing 0.001 EU/mL.

Underlying sensing principle

The enhanced performance observed with the αBET™ arises from the way that transmitted light is observed and quantified. In a typical turbidimetric assay light is passed through a sample and the difference between incident and transmitted determined. This is also the

Figure 1: The CMD αBET™ system

case for the αBET™ turbidimetric assay. However, rather than simply measuring the difference in intensity of incident and transmitted light, αBET™ utilises a proprietary magneto-optical sensing technology that uses polarising optics to couple incident light with inert magnetic nanorod reporters to induce a modulation in the light transmitted through the reaction mixture. The αBET® system measures changes in the amplitude of the induced modulation to monitor the progress of the turbidimetric LAL reaction with far greater sensitivity than offered by conventional plate, tube or cartridge readers.

Performance of the system:

Extensive studies have been undertaken to demonstrate that the performance of the αBET™ system is substantially equivalent in terms of accuracy and precision to existing tube reader based LAL assays. Figure 2 shows the results of a series of calibrations run on the CMD αBET™ system

and the FUJIFILM Wako Toxinometer® tube reader. On both systems, three different lots of Pyrostar™ ES-F 0.015 EU/mL sensitivity lysate was used (n = 4 for each lot). On the αBET™ system, each lot of lysate was tested in combination with three different batches of CMD reagent (n = 4 for each combination of reagents). Data is presented for each lot of Pyrostar™ tested (Figures 2a and 2c) as well as a combined calibration including data from all lots (Figures 2b and 2d). As is evident from Tables 1 and 2, the performance of the two systems is comparable despite the time to result being dramatically reduced for the αBET™; the % CV for each concentration tested sits between 5.9% and 7.7% across the two systems with the correlation coefficient (r) for all calibrations exceeding the regulatory stipulated threshold of ≥ 0.980.

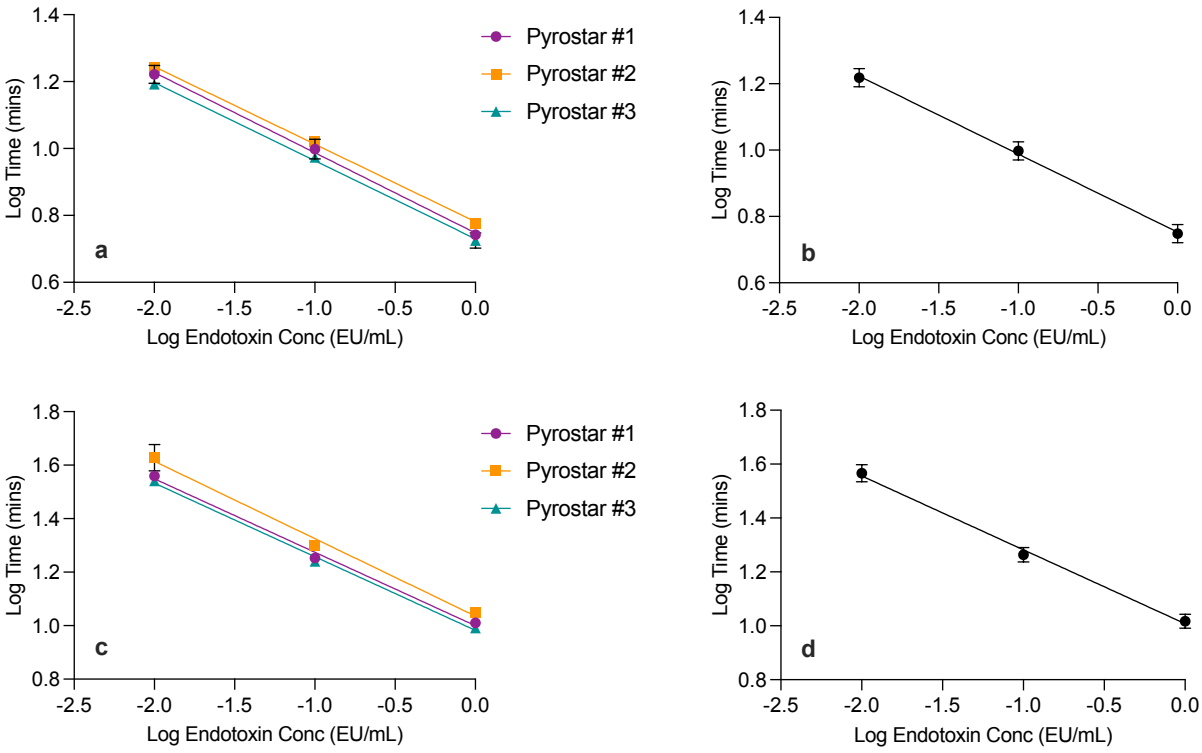

*Figure 2: Three-point calibrations performed on the CMD αBET™ system (**a** and **b**) and FUJIFILM Wako Toxinometer® (**c** and **d**). Three lots of Pyrostar™ ES-F lysate were evaluated on both systems. On the αBET™ system, each lot of lysate was combined with three different batches of CMD nanorod reagent. Individual calibrations are shown in a and c, whilst b and d show the calibration generated by combining all data across reagent lots for the αBET™ and Toxinometer® systems respectively. Data is plotted as mean ± standard deviation, n = 4 per lot of Pyrostar™ ES-F / reagent combination.*

Table 1: Summary of calibration data produced using the CMD αBET™ and FUJIFILM Wako Toxinometer® systems. Mean times are calculated from all Pyrostar™ ES-F / CMD nanorod reagent combinations.

CMD αBET™ System			
Endotoxin conc. (EU/mL)	**Mean time (mins)**	**St Dev**	**% CV**
1	5.6	0.36	6.4
0.1	10.0	0.63	6.3
0.01	16.6	1.04	6.3
FUJIFILM Wako Toxinometer® tube reader			
Endotoxin conc. (EU/mL)	**Mean time (mins)**	**St Dev**	**% CV**
1	10.5	0.62	5.9
0.1	18.5	1.15	6.2
0.01	37.2	2.88	7.7

Table 2: Correlation coefficient, r, values for the individual and combined calibrations generated on the CMD αBET™ and FUJIFILM Wako Toxinometer® systems.

	CMD αBET™ System	**FUJIFILM Wako Toxinometer®**
Pyrostar™ lot	**Correlation coefficients, r**	
#1	0.997	0.992
#2	0.991	0.997
#3	0.998	0.996
Combined	0.991	0.990

The αBET™ system has also demonstrated good performance when challenged with a range of pharmaceutical samples. Test for interferences, as described in the harmonised pharmacopoeial texts, were performed for 10 different samples. Samples were spiked with control standard endotoxin to produce final concentrations of 0.1 EU/mL. Spiked and unspiked controls were analysed in duplicate on both the αBET™ and Toxinometer® systems without any predilution. Results are presented in Table 3. Calculated recoveries from all samples fell within the regulatory accepted range of 50 – 200% on the αBET™ system, with only the Ringers solution falling outside of this range when tested on the Toxinometer®. All samples were associated with a % CV of < 10%.

The system has also been shown to work with complex bio-pharmaceutical samples and with nanoparticle formulations. When compared to traditional turbidimetric and chromogenic assays, the αBET™ is less impacted by sample colour and haziness.

Table 3: Endotoxin recovery from 10 different pharmaceutical samples. Mean % recovery along with %CV are provided for all samples.

Sample	αBET™		Toxinometer®	
	Recovery (%)	%CV	Recovery (%)	%CV
Sodium chloride 0.9%	61	6.2	99	2.8
Sodium chloride 3%	118	3.1	107	8.4
Glucose 2.5%	72	0.5	105	5.7
Ringers solution	181	1.0	208	0.0
Magnesium sulfate 50 mM	65	3.6	82	7.8
Sodium bicarbonate 0.25%	87	1.3	86	0.0
Sodium citrate 0.1%	77	7.3	132	3.0
Sodium heparin 1 U/mL	63	1.4	97	5.5
Xylitol 2.5%	60	8.6	103	2.8
Sodium ascorbate 25 mg/mL	109	5.2	124	5.9

Technical specifications

αBET™ Instrument (Product no. CMD-A-0007-EN)	
Dimensions	(W) 243 mm, (D) 290 mm, (H) 215 mm
Weight	3.0 kg
Power supply	Mains supply: 100-240 VAC, 50/60 Hz, switch mode power supply unit. 24VDC-1A (24W)
Display	7 in Touchscreen
LAN interface	RJ45 connector
Serial input	DB9 connector
USB	Micro USB B
αBET™ Consumable pack (Product no. CMD-A-0006-C)	
Contents	25 x disposable cartridges 5 x CMD nanorod reagent vials
Pyrostar™ ES-F lysate – available from FUJIFILM Wako Chemicals USA Corp.	

Cotton Mouton Diagnostics Ltd
Unit 8 Cae Gwyrdd
Greenmeadow springs
Cardiff
www.cm-dx.com