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Optimization of Endotoxin Testing for Cell & Gene Therapy Products

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INTRODUCTION

In the field of cell and gene therapy, some of the newest drugs use whole cells or genetic materials to treat diseases. However, testing these drugs for endotoxins can be challenging because endotoxins tend to bind to the drug components. bioMérieux offers a solution called ENDOLISA®, which uses a special phage protein attached to a plate to effectively remove endotoxins from such samples. This enables subsequent testing at very low dilutions, typically 1:5 or 1:10. After endotoxin binding, the sample is washed away, leaving an empty plate, which is then tested using the regular rFC fluorescent method.

We have tested ENDOLISA with common interfering substances, and you can see the recoveries in Table 1. Compare these recoveries with those achieved using LAL and rFC methods with ENDOLISA.

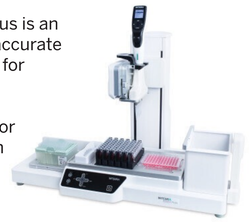
Several companies have adopted this method for drug release testing. Validation data indicates excellent PPC recovery (80-120%) and recovery of endotoxins spiked into the sample solution (80-100%). This "hard spike" is crucial to demonstrate that if endotoxins were present, they would be recoverable. In contrast, when using LAL testing, analysts are limited in their ability to make the solution suitable for endotoxin recovery, sometimes leading to challenges.

MATERIALS & METHODS

The steps (in dark green) are more efficient when using robotic automation, which saves time and ensures optimal performance.

The Integra® Assist Plus is an affordable yet highly accurate robotic platform ideal for ENDOLISA®.

The robot is suitable for "walk-away" operation until it's time to add the plate to the reader.



Assist Plus pipetting robot from INTEGRA®

Current ENDOLISA

- Standard Reconstitution
- Preparation of the Standard Curve
- Sample preparation
- Filling the plate with standards and samples
- Addtion of the PPC
- Addition of Binding Buffer
- Incubation/Binding step
  - Incubator: 37°C with shaking for 90 min.
- Washing step
  - 3 manual washing cycles in the sink (!)
- Assay mix preparation
- Addition of the assay mix to the plate
- Measurement
  - Synergy HTX

ENDOLISA with Assist Plus

- Standard Reconstitution
- Preparation of the Standard Curve
- Sample preparation
  - Could be done by Assist Plus in principle
- Filling the plate with standards and samples
- Addtion of the PPC
- Addition of Binding Buffer
- Incubation/Binding step
  - No separate incubator needed. Incubation on the Assist Plus: Incubator: 37°C with shaking for 90 min.
- Washing step
  - Washing step done by Assist Plus (or Viaflo 96)
- Assay mix preparation
- Addition of the assay mix to the plate
- Measurement
  - Synergy HTX

ENDOLISA® METHOD

Table 1. Comparison between ENDOLISA and LAL assays shows the highest tolerated concentration of substances for valid LPS spike recovery.

Table 1 | Highest tolerated concentrations of substances for valid LPS spike recovery, a comparison between EndoLISA and LAL assay.

	Substance	Solvent	*EndoLISA*	*LAL assay*
Buffer/pH	Acetate (pH 4.0)	100 mM NaCl	50 mM	12.5 mM
	Acetate (pH 5.0)	100 mM NaCl	100 mM*	12.5 mM
	MES (pH 6.0)	100 mM NaCl	100 mM*	5 mM
	Potassium phosphate (pH 7.2)	100 mM NaCl	100 mM*	50 mM
	Imidazole (pH 7.4)	Water	500 mM	40 mM
	HEPES (pH 7.5)	100 mM NaCl	100 mM*	100 mM*
	Sodium borate (pH 8.0)	100 mM NaCl	100 mM*	50 mM
Salt	NaCl	Water	1 M	0.5 M
	KCl	Water	1 M	0.25 M
Chaotropic agent	Urea	Water	6 M	0.5 M
	Guanidinium chloride	Water	1 M	0.05 M
Organic solvent	Methanol	Water	20%*	5%
	Ethanol	Water	30%	0.5%
	2-Propanol	Water	20%	0.2%
	DMSO	Water	10%	2%
Detergent	SDS	Water	0.05%	0.001%
	CTAB	Water	0.004%	0.0001%
	Zwittergent 3-14	Water	0.02%	0.005%
	Tween 20	Water	2%	0.1%
	Triton X-100	Water	0.02%	0.005%
Chelator	EDTA (pH 8.0)	Water	0.4 mM	0.4 mM
	Citrate (pH 7.5)	Water	10 mM	10 mM
Protease inhibitor	Benzamidine	Water	100 mM*	0.1 mM
	PMSF	2-Propanol	5 mM	<0.05 mM
Antibiotic	Rifampicin	Methanol	3.5 mg/ml	0.04 mg/ml
	Chloramphenicol	Ethanol	3.5 mg/ml	0.1 mg/ml

*Highest concentration tested.

Reference: Overcoming common interference properties in Limulus testing using ENDOLISA®. See: Grallert, H., Leopoldseder, S., Schuett, M. et al. ENDOLISA®: a novel and reliable method for endotoxin detection. Nat Methods 8, iii–v (2011).

DATA

Tables 2, 3 and 4 below show the BET sample validation criteria achieved using ENDOZYME II. This includes excellent PPC recoveries as well as sensitive LOD.

Table 2. Shows BET validation criteria for Cell Culture Media sample types tested by ENDOZYME II.

Sample Type	Sample ID	Dilution	LOD [EU/mL]	Solvent	PPC [%]	Measured Endotoxin activity [EU/mL]
Prime-XV Media	Lot 1	1:10	0.5	WEF	104	< LOD
		1:20	1.0		95	< LOD
	Lot 2	1:10	0.5		134	< LOD
		1:20	1.0		93	< LOD
	Lot 3	1:10	0.5		102	< LOD
		1:20	1.0		113	< LOD
TexMACS Media	Lot 1	1:10	0.5	WEF	102	< LOD
	Lot 2	1:10	0.5		99	< LOD
	Lot 3	1:10	0.5		118	< LOD

Table 3. Shows BET validation criteria for Cell Culture Media sample type tested by ENDOZYME II GO.

ENDOZYME® II GO

Sample Type	Sample ID	Dilution	LOD [EU/mL]	Solvent	PPC [%]	Measured Endotoxin activity [EU/mL]
TexMACS Media	Lot 1	1:10	0.05	WEF	72	< LOD
		1:20	0.10		87	< LOD

DATA

Table 4. Show data from CAR-T Cell, T-Cell Product and Extra Cellular Vesicle Matrix samples tested by ENDOZYME II GO.

Sample Type	Sample ID	Hard spike	Dilution	LOD [EU/mL]	Solvent	PPC [%]	Hard spike recovery [%]	Measured Endotoxin activity[EU/mL]
CAR-T-Cell	Patient 1	-	1:40	2.0	WEF	119	-	< LOD
	Patient 1	5 EU/mL	1:40	2.0		126	80.0	0.100
	Patient 2	-	1:40	2.0	WEF	111	-	< LOD
	Patient 2	5 EU/mL	1:40	2.0		106	57.6	0.072
	Patient 3	-	1:40	2.0	WEF	122	-	< LOD
	Patient 3	5 EU/mL	1:40	2.0		135	89.6	0.112
	Patient 4	-	1:40	2.0	WEF	124	-	< LOD
	Patient 4	5 EU/mL	1:40	2.0		130	88.8	0.111
	Patient 5	-	1:40	2.0	WEF	122	-	< LOD
	Patient 5	5 EU/mL	1:40	2.0		118	84.8	0.106
T-Cell Product	Product 1, Lot 1	-	1:5	0.25	WEF	122	-	< LOD
			1:10	0.5		118	-	< LOD
	Product 1, Lot 1	-	1:5	0.25	WEF	85	-	< LOD
			1:10	0.5		81	-	< LOD
	Product 1, Lot 1	0.8 EU/mL	1:5	0.25	WEF	84	56.2	0.0899
			1:10	0.5		66	82.0	0.0656
	Product 1, Lot 2	-	1:5	0.25	WEF	70	-	< LOD
			1:10	0.5		63	-	< LOD
	Product 1, Lot 2	-	1:5	0.25	WEF	70	-	< LOD
			1:10	0.5		63	-	< LOD
	Product 1, Lot 3	-	1:5	0.25	WEF	77	-	< LOD
			1:10	0.5		74	-	< LOD
	Product 2, Lot 1	-	1:5	0.25	WEF	92	-	< LOD
			1:10	0.5		78	-	< LOD
	Product 2, Lot 1	-	1:5	0.25	WEF	99	-	< LOD
			1:10	0.5		84	-	< LOD
	Product 2, Lot 1	0.8 EU/mL	1:5	0.25	WEF	92	76.9	0.123
			1:10	0.5		68	97.4	0.078
	Product 2, Lot 2	-	1:5	0.25	WEF	102	-	< LOD
			1:10	0.5		110	-	< LOD
	Product 2, Lot 3	-	1:5	0.25	WEF	80	-	< LOD
			1:10	0.5		82	-	< LOD
Extra Cellular Vesicle Matrix	Product 1 Lot 1	-	1:10	0.5	WEF	122	-	< LOD
			1:50	2.5		118	-	< LOD
	Product 1 Lot 1	5 EU/mL	1:10	0.5	WEF	130	78.9	0.395
			1:50	2.5		123	96.5	0.096
	Product 1 Lot 2	-	1:10	0.5	WEF	183	-	< LOD
			1:50	2.5		152	-	< LOD
	Product 1 Lot 3	-	1:10	0.5	WEF	180	-	< LOD
			1:50	2.5		170	-	< LOD
	Product 1 Lot 4	-	1:10	0.5	WEF	166	-	< LOD
			1:50	2.5		158	-	< LOD
	Product 1 Lot 5	-	1:10	0.5	WE F	200	-	< LOD
			1:50	2.5		165	-	< LOD
	Product 1 Lot 6	-	1:10	0.5	WEF	135	-	< LOD
			1:50	2.5		210	-	< LOD
	Product 2 Lot 1	-	1:5	0.25	WEF	77	-	< LOD
			1:10	0.5		106	-	< LOD
	Product 2 Lot 2	-	1:5	0.25	WEF	99	-	< LOD
			1:10	0.5		111	-	< LOD
	Product 2 Lot 3	-	1:5	0.25	WEF	59	-	< LOD
			1:10	0.5		76	-	< LOD
	Product 2 Lot 4	-	1:5	0.25	WEF	131	-	< LOD
			1:10	0.5		160	-	< LOD
	Product 2 Lot 5	-	1:5	0.25	WEF	34	-	< LOD
			1:10	0.5		81	-	< LOD

CONCLUSION

While conventional Limulus-based tests work well for some cell and gene therapy products, others exhibit poor recovery with LAL/rFC. ENDOLISA® offers a solution by removing interfering factors before endotoxin testing, as evidenced by the excellent PPC recoveries (80-120%).

The Importance of the "Hot Spike" - Sometimes a user (performing validation) may not be aware of the necessity of a hard spike and alternatively the PPC that is spiked into the plate as a 10 microliter spike is much more easily recovered than the hard spike and does not really mimic endotoxin that has been thoroughly mixed in the drug sample matrix. This extra sample-reagent interaction is where the deleterious binding occurs most. Thus, validation is not just getting something to work, but rather scientifically proving that the endotoxin in a sample will truly be detected in an outgoing quality control (QC) test sample.

PRN XXXXXX RevGLA