

CHECKLIST FOR BACTERIAL ENDOTOXIN TEST (BET)

Version: June 2022



VALIDATION TEST REQUIREMENT	
1. Confirmation of Labeled Lysate Sensitivity (Gel Clot) / Standard Curve (Photometric Method)	
Method	<p>Gel Clot Method:</p> <ul style="list-style-type: none"> At least 4 concentration of standard endotoxin (2 λ, λ, 0.5 λ, 0.25 λ) 4 replicates Geometric Mean of End Point = 0.5 λ - 2 λ <p>Photometric Method: For the generation of standard curve, following information is required:</p> <ul style="list-style-type: none"> 3 endotoxin concentration to generate standard curve 3 replicates for each concentration correlation coefficient (r) must be ≥ 0.98 (linear graph must be demonstrated) Standard curve is required
Result (raw data)	<p>Gel Clot Method:</p> <ul style="list-style-type: none"> Geometric Mean of End Point = 0.5 λ - 2 λ <p>Photometric Method:</p> <ul style="list-style-type: none"> Standard Curve following the criteria set in Method.
2. Test for Interfering Factor (Gel Clot/ Photometric Method)	
Method	<p>3 batches of data is required</p> <p>Gel Clot Method:</p> <ul style="list-style-type: none"> Detailed method for Test for Interfering Factor A: sample only - 4 replicates B: sample + endotoxin (2λ or 4 different λ concentration) - 4 replicates C: LAL water + endotoxin (4 different λ concentration) - 2 replicates D: LAL water only - 2 replicates <p>Photometric Method:</p> <ul style="list-style-type: none"> Detailed method for Test for Interfering Factor A: sample only – not less than 2 replicates B: sample + endotoxin (middle concentration of the standard curve) – not less than 2 replicates C: LAL water + endotoxin (at least 3 concentration) – each concentration not less than 2 replicates D: LAL water only – not less than 2 replicates
Result (raw data)	<p>Gel Clot Method:</p> <ul style="list-style-type: none"> A and D must be negative Geometric Mean of End Point = 0.5 λ - 2 λ <p>Photometric Method:</p> <ul style="list-style-type: none"> PPC Recovery between 50% - 200%
3. MVD Calculation & ELC Calculation (Gel Clot Method /Photometric Method)	
Method	Calculation of MVD or ELC (if applicable) (Formula)
Result (raw data)	<ul style="list-style-type: none"> Actual calculation Product specific

ROUTINE TEST REQUIREMENT	
1. Limit test/Semiquantitative (Gel Clot Method /Photometric Method)	
Method	<p>3 batches of data is required</p> <p>Gel Clot Method:</p> <ul style="list-style-type: none"> • Acceptance criteria • Protocol of analysis for bacteria endotoxin test (routine test procedure) • A: sample only - 2 replicates • B: sample + endotoxin (2λ concentration) - 2 replicates • C: LAL water + endotoxin (2λ concentration) – 2 replicates • D: LAL water only - 2 replicates <p>Photometric Method:</p> <ul style="list-style-type: none"> • Protocol of analysis for bacteria endotoxin test (routine test procedure) • Acceptance criteria
Result (raw data)	Not Required
2. Preparation of Reagents, Endotoxin Standard & Sample (Gel Clot Method /Photometric Method)	
Method	<p>Reagents</p> <ul style="list-style-type: none"> • Lysate • Endotoxin <p>Endotoxin Standard</p> <ul style="list-style-type: none"> • At least 4 concentrations • Procedure of serial dilution (How the serial dilution performed) <p>Sample</p> <ul style="list-style-type: none"> • Must be specific to product • Dilution, pH adjustment, additives (e.g. Pyrosperser™ (Dispersing Agent), MgCl₂ etc)
Result (raw data)	Not Required
3. MVD Calculation & ELC Calculation (Gel Clot Method /Photometric Method)	
Method	<ul style="list-style-type: none"> • Calculation of MVD or ELC (if applicable) • Actual calculation • Product specific
Result (raw data)	Not Required
4. CoA for Lysate and Endotoxin (Gel Clot Method / Photometric m Method)	
	CoA for Lysate and Endotoxin is required
5. List of apparatus and reagents (Gel Clot Method / Photometric m Method)	
	<ul style="list-style-type: none"> • List/ Certificate of depyrogenated of glasswares • List of reagents used • LAL water/WFI, endotoxin, Lysate, pH adjustor (buffer/acid/base)

