

## CHECKLIST FOR BACTERIAL ENDOTOXIN TEST (BET)

Validation test		
1.	Confirmation of Labeled Lysate Sensitivity (Gel Clot) / Standard Curve (Photometric Method)- 1 Batch of Endotoxin	<p style="text-align: center;"><b>Method</b></p> <p><b>Gel Clot Method</b> must have these following informations:-</p> <ul style="list-style-type: none"> <li>- 1 batch of lysate</li> <li>- At least 4 concentration of standard endotoxin (2 λ, λ, 0.5 λ, 0.25 λ)</li> <li>- 4 replicates</li> <li>- Geometric Mean of End Point= 0.5 λ - 2 λ</li> </ul> <p><b>Photometric Method:</b> For the generation of standard curve applicant must provide the following information*:</p> <ul style="list-style-type: none"> <li>- 3 endotoxin concentration to generate standard curve</li> <li>- 3 replicates for each concentration</li> <li>- correlation coefficient (r) must be ≥ 0.98 (linear graph must be demonstrated)</li> </ul>
		<p style="text-align: center;"><b>Result (raw data)</b></p> <p><b>Gel Clot:</b></p> <ul style="list-style-type: none"> <li>- Geometric Mean of End Point= 0.5 λ - 2 λ</li> </ul> <p><b>Photometric:</b></p> <ul style="list-style-type: none"> <li>- Standard Curve following the criteria set in Method.</li> </ul>
2.	Test for Interfering Factor (Gel Clot/ Photometric Method)- 3 Batches of finished product	<p style="text-align: center;"><b>Method</b></p> <p><b>Gel Clot Method</b> must have these following informations:-</p> <ul style="list-style-type: none"> <li>- Detailed method for Test for Interfering Factor</li> <li>- 3 batches of finished product</li> <li>- A: sample only - 4 replicates</li> <li>- B: sample + endotoxin (2λ or 4 different λ concentration)- 4 replicates</li> <li>- C: LAL water + endotoxin (4 different λ concentration) - 2 replicates</li> <li>- D: LAL water only - 2 replicates</li> </ul> <p><b>Photometric Method:</b></p> <ul style="list-style-type: none"> <li>- PPC Recovery must be between 50% - 200%</li> </ul>
		<p style="text-align: center;"><b>Result (raw data)</b></p> <p><b>Gel Clot:</b></p> <ul style="list-style-type: none"> <li>- Raw data following the criteria set in Method for 3 batches of finished product.</li> <li>- A and D must be negative</li> <li>- Geometric Mean of End Point= 0.5 λ - 2 λ</li> </ul> <p><b>Photometric:</b></p> <ul style="list-style-type: none"> <li>- PPC Recovery between 50% - 200%</li> </ul>
3.	MVD Calculation & ELC Calculation (if applicable) (Gel Clot/Photometric Method)	<p style="text-align: center;"><b>Method</b></p> <ul style="list-style-type: none"> <li>- Calculation of MVD or ELC (if applicable) (Formula)</li> </ul>
		<p style="text-align: center;"><b>Result (raw data)</b></p> <ul style="list-style-type: none"> <li>- Actual calculation</li> <li>- Product specific</li> </ul>

Routine Test		
1.	COA for Lysate and Endotoxin (Gel Clot/ Photometric Method)	
2.	List of Apparatus and Reagents (Gel Clot/ Photometric Method)	
3.	Preparation of Reagents, Endotoxin Standard and Sample. (Gel Clot/ Photometric Method)	<p style="text-align: center;"><b>Method</b></p> <ul style="list-style-type: none"> <li>- Detailed procedure for preparation of reagent, endotoxin standard and sample</li> <li>- Steps for dilution wherever applicable</li> </ul>
		<p style="text-align: center;"><b>Result (raw data)</b></p> <ul style="list-style-type: none"> <li>- Actual dilution records.</li> </ul>
4.	MVD Calculation & ELC Calculation (if applicable) (Gel Clot/Photometric Method)	<p style="text-align: center;"><b>Method</b></p> <ul style="list-style-type: none"> <li>- Calculation of MVD or ELC (if applicable) (Formula)</li> </ul>
		<p style="text-align: center;"><b>Result (raw data)</b></p> <ul style="list-style-type: none"> <li>- Actual calculation</li> <li>- Product specific</li> </ul>
5.	Limit Test /Semiquantitative Test (Gel Clot/ Photometric Method)	<p style="text-align: center;"><b>Method</b></p> <p><b>Gel Clot Method</b> must have these following informations:-</p> <ul style="list-style-type: none"> <li>- Detailed procedure on how to perform routine bacteria endotoxin testing.</li> <li>- 3 batches of finished product</li> <li>- A: sample only – 2 replicates</li> <li>- B: sample + endotoxin (2λ concentration) – 2 replicates</li> <li>- C: LAL water + endotoxin (2λ concentration) – 2 replicates</li> <li>- D: LAL water only – 2 replicates</li> <li>- A and D must be negative</li> <li>- B and C must be positive</li> </ul> <p><b>Photometric Method:</b></p> <ul style="list-style-type: none"> <li>- Detailed procedure on how to perform routine bacteria endotoxin testing.</li> <li>- Sample must meet limit specified and PPC Recovery must be between 50% - 200%</li> </ul>
		<p style="text-align: center;"><b>Result (raw data)</b></p> <p><b>Gel Clot:</b></p> <ul style="list-style-type: none"> <li>- Raw data following the criteria set in Method for 3 batches of finished product.</li> <li>- A and D must be negative</li> <li>- B and C must be positive</li> </ul> <p><b>Photometric Method</b></p> <ul style="list-style-type: none"> <li>- Sample meet the limit specified.</li> <li>- PPC Recovery between 50% - 200%</li> </ul>

Updated : 5<sup>th</sup> September 2017