BACTERIA ENDOTOXIN TEST (BET) VALIDATION

Product Name/Protocol No.			
Method Used	Gel Clot LimitTest / Gel Clot Semi-quantitative Test		

Subject	Acceptance Criteria	Details Provided by Company	Comments
Protocol of Analysis			
CoA	- check endotoxin level & pH - Endotoxin level in USP/BP/EP - Provide the CoA for lysate & endotoxin (parallel batch)		
List of apparatus and reagents	- Depyrogenation of glasswares - List of reagents used - LAL water/WFI, endotoxin, Lysate, pH adjustor (buffer/acid/base)		
Preparation of Reagents	- Lysate - Endotoxin		
Preparation of Endotoxin Standard	- At least 3 concentrations - How the serial dilution performed		
Preparation of Sample	- Must be specific to product - Dilution, pH adjustment, additives (e.g. pyrosperse, MgCl2 etc)		
MVD calculation	- Real calculation, not formula $MVD = \dfrac{EL \times Conc}{\lambda}$		
ELC Calculation (if applicable)	- Real calculation, not formula $ELC = K/D \times Conc$		
Validation - method (can be in Protocol of Analysis or in the Validation section) and raw data - raw data can be handwritten or computerized - but must have signature of analyst			
i. Preparatory Testing			
Confirmation of labeled lysate sensitivity (Gel Clot)	-1 batch of lysate - at least 4 concentration of std (2 λ, , λ 0.5 λ, 0.25 λ) - 4 replicates - Geom. Mean of end point = 0.5 λ - 2 λ)		

Test for Interfering Factors	3 batches of finished product A: sample only - 4 repl B: sample + endot (2λ or 4 different λ conc.) - 4 repl C: LAL water + end (4 different λ conc.) - 2 repl D: LAL water only - 2 repl - A and D must be -ve - Geom. Mean of end point = 0.5 λ - 2 λ)		
Non Inhibitory Dilution /Non Interfering Conc	2 duplicates 4 concentrations (min)		
ii. Test			
Limit Test or Semi Quantitative Test (aka Inhibition/Enhancement Test)	3 batches of finished product **i) Limit Test** A: sample only – 2 repl B: sample + endot (2λ conc) – 2 repl C: LAL water + endot (2λ conc) – 2repl D: LAL water only – 2 repl - A and D must be –ve - B and C must be +ve		