GEL– CLOT VALIDATION METHOD

1. General

New operators must be adequately trained by a competent staff member and perform a satisfactory Operator Verification, (**Form 590**), prior to performing Gel-Clot method validations. Gel-clot method validations may be performed once all training requirements are met and have been deemed to be satisfactory following review.

The gel clot validation method for Bacterial Endotoxin testing described in this SOP, is to determine the level of Inhibition/Enhancement of products on the LAL test for endotoxins within the allowable Maximum Valid Dilution (MVD) for each type of product. The Gel-Clot techniques detect or quantify endotoxins based on clotting of the LAL reagent in the presence of endotoxin.

To be determined for each type of product, using the highest and lowest concentration of active. If either concentration shows inhibition or enhancement, then each remaining concentration must be tested. At least three (3) Production batches of each finished product should be tested for inhibition and enhancement.

1.1. Materials required:

10ml sterile disposable pipettes

1ml sterile disposable pipettes

Sterile disposable micropipettes

Pyrogen-free water for injection (WFI)

10mm x 75mm test tubes

10ml depyrogenated test tubes

20ml depyrogenated test tubes

E.coli endotoxin

Pyrogent

Pyrospers

Vortex mixer

Micro pipettor

Heating Block.

2. Preliminary Inhibition and Enhancement Testing:

Preliminary Inhibition/Enhancement testing consists of running two (2) sets of product dilutions in parallel, one set containing product alone, the other set containing endotoxin spiked product such that the concentration of the product decreases with increasing dilution while the concentration of endotoxin remains the same. The final concentration of the endotoxin spike should be equal to twice the sensitivity of the Lysate used.

2.1. Method:

Preparation of Pyrogent, see **MICLAB 080.**

Preparation of the Endotoxin Standard, see **MICLAB 080.**

- 12 tubes Product compatibility
- 2 tubes Negative controls
- 8 tubes Positive controls

Immediately swirl gently to mix and incubate undisturbed at 37°C ± 0.5°C in the heating block for 60 ± 2 minutes.

After 60 minutes incubation, gently remove the tubes from the block and rotate them through 180 degrees.

Test results

Positive: Formation of a firm gel that maintains its integrity when the test tube is inverted.

Negative: Total absence of gel, or the formation of a viscous gel that does not maintain its integrity when inverted.

Note: The results sheet - Preliminary Inhibition/Enhancement Test is to be filled in.

3. Interpretation of results of Preliminary Inhibition/Enhancement test

The results of the Product Control Dilution series and the Product Compatibility series are to be compared.

a) The Product Control Dilution series should show no clotting (positive reaction) if the product contains no endogenous endotoxin.

b) The first tube in the Product Compatibility Dilution series that does clot, i.e. a positive reaction is the dilution of product required to overcome inhibition. If no positive reactions are recorded, further dilution of the product is required to overcome inhibition. Repeat the test using further dilution steps in 3(b)(1) and 3(c)(1).

3.1. Calculations for the Maximum Valid Dilution and the Endotoxin Limits

The Maximum Valid Dilution (MVD) must be calculated.

When a product is diluted to overcome interference or inhibition, any endotoxin present is also diluted.

The MVD is the degree to which a product can be diluted before the sensitivity of the assay method to detect the diluted endotoxin concentration is exceeded.

The MVD is equal to the endotoxin limit divided by Lambda

Lambda (λ) is equal to the label claim for the gel clot lysate or the lowest standard for the KCA method.

The MVD is the maximum dilution that must not be exceeded for routine product testing.

Examples of MVD Calculations:

GEL-CLOT	KCA
Lysate sensitivity = 0.06 EU/mL	Lowest Standard = 0.005EU/mL
Endotoxin Limit for Product = 3.0EU/mL	Endotoxin Limit for Product = 3.0EU/mL
MVD = 3.0EU/mL / 0.06EU/mL	MVD = 3.0EU/mL / 0.005EU/mL
MVD = 50	MVD = 600

Please note that the higher sensitivity of the KCA method will give a larger MVD. This, in turn, will allow a greater dilution to overcome any inhibition that may be present.

The **Endotoxin Limit** is determined by:
(1) specific monograph for that product (BP, EP, USP or JP),
(2) by regulatory requirement (local or export)
(3) corporate product specification or 4) by calculation.

Verification Assay Result Sheet

Verification Assay For Microbiology Laboratory Technicians
(Ref. MICLAB 105)

Verification Assay Date: _____

Name of Technician: _____

Test Reagents

Reagents	Lot No.	Reconstitution Date	Expiry date	
Pyrogent				EU/mL sensitivity
Endotoxin				EU/mL potency
Pyrosperse		NA		2% working concentration
Test kit		NA		

L.A.L, Endotoxin & Endotoxin Working Standards diluent.
Any sterile batch (WFI) (Tested to be L.A.L. negative) Batch No.: Expiry:

Test Session Standards - Results
Key: (+) firm gel, (-) no gel or viscous gel.

Replicate Assay Number	Endotoxin Concentration & Gelation Results (EU/mL)							Endpoint	
	1	0.5	0.25	0.125	0.06	0.03	0.015	EU/mL	Log_{10}

Negative Controls
Key: (+) firm gel, (-) no gel or viscous gel.

Replicate Assay No.	Control Results
1	
2	

Bacterial Endotoxin Test Data

(Ref. MICLAB 105)

Test Article:

Product Name:	
Batch No.:	
Container And Size:	
No. Of Samples:	
Date Received:	

Test Reagents

LAL Pyrogen Plus Test Kit	BioWhittaker (brand)	Lot No.:	_____
LAL: Pyrogent	BioWhittaker (brand)	Lot No.:	_____
		Expiry Date:	_____
		Labelled Sensitivity:	_____EU/ml
LAL diluent	Any batch of WFI	Batch No:	
		(tested to be LAL neg)	_____

Pyrogent Reconstitution Date: _____

Endotoxin Control Standard (CSE)	BioWhittaker (brand)	Lot No:	_____
		Expiry Date:	_____
		Labelled Potency:	_____EU/ml
Endotoxin Diluent	Any batch of WFI	Batch No.:	_____
		(tested to be LAL neg)	

Endotoxin Reconstitution Date:_____

Endotoxin Working Standards Diluent	Any batch of WFI	Batch No.	_____
		(tested to be LAL neg)	
Dispersing Agent	BioWhittaker (brand) Pyrosperse	Lot No.:	_____
		Expiry Date:	_____
		Working conc.	_____2%_____

Bacterial Endotoxin Gel Clot Validation

Final Inhibition and Enhancement Test
(Ref. MICLAB 105)

Result sheet – Bacterial Endotoxin test (U.S.P.) Pyrogent

Interpretation of Results:

Test		Result	Acceptance Levels
A2	Product Endotoxin Dilution series endpoint, Part 1 (Highest dilution positive)		=_____EU/mL (0.5 – 2 x Lysate sensitivity)
B2	Positive Controls, Part 2 (Highest dilution positive)		=_____EU/mL (0.5 – 2 x Lysate sensitivity)
C2	Negative controls, Part 3	Pos / Neg	Must be negative.
D2	Lowest dilution giving positive Lysate in Preliminary test. (Product Compatibility Test, B1 result)		Less than MVD.
E2	Comparison of Endotoxin determinations in Product A2and Water B2. (ie Dilution level they differ by)		Must not differ by more than plus or minus a 2 fold dilution.

Have all the acceptance levels been met? YES / NO

