

Standard Operating Procedure

Title: Gel Clot Validation Method

- 2 tubes Negative controls
- 8 tubes Positive controls

Immediately swirl gently to mix and incubate undisturbed at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ 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.

- The Product Control Dilution series should show no clotting (positive reaction) if the product contains no endogenous endotoxin.
- 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 = $\frac{3.0\text{EU/mL}}{0.06\text{EU/mL}}$	MVD = $\frac{3.0\text{EU/mL}}{0.005\text{EU/mL}}$
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.

The Endotoxin Limit = (K/D) x Potency