Schematic for Test for Interfering Factors Using Pyrosate® PPC Vials

1. The USP Bacterial Endotoxin Test chapter requires the Test for Interfering Factors be performed in duplicate for endotoxin in water (LRW) and in quadruplicate for endotoxin diluted in sample.

2. Set Up (Endotoxin in LRW; Standard Curve)
   a. Set up two racks of SPL and PPC vials in duplicate according to diagram below.

   ![Diagram of racks](image)

   **RACK 1** – Reconstitution Rack
   **RACK 2** – Incubation Rack

3. Preparation
   a. Use a new pipette (or tip) for each transfer/removal combination.
   b. Remove stoppers taking care not to contaminate the vials and test per instructions below.

4. Testing
   a. Incubate Rack 2 in a water bath equilibrated at 37 ± 1°C for the specified incubation time (± 1 minute).
   b. At the end of the incubation time, read the test by inverting each vial in one smooth motion starting with the negative control vials then ½λ vials, etc.

   ![Diagram of testing](image)

   **RACK 2** – Incubation Rack

   **Positive test**
   If a firm gel forms that withstands inversion, the test is scored as positive (+). All other results are negative (-), even if it is clear that a gel has formed but the clot breaks.
5. Set Up (Endotoxin diluted in sample)
   a. Set up two racks of SPL and PPC vials in quadruplicate according to diagram below.
   
6. Preparation
   a. Sample corresponds to the dilution which during Sample Characterization did not cause inhibition and which did not contain endotoxin.
   b. Use a new pipette (or tip) for each transfer/removal combination.
   c. Remove stoppers taking care not to contaminate the vials and test per instructions below.
7. Testing
   a. Incubate Rack 4 in a water bath equilibrated at 37 ± 1°C for the specified incubation time (± 1 minute).

   b. At the end of the incubation time, read the test by inverting each vial in one smooth motion starting with the negative control vials then ¼ λ vials, etc.

   If a firm gel forms that withstands inversion, the test is scored as positive (+). All other results are negative (−), even if it is clear that a gel has formed but the clot breaks.

8. Interpretation of Results of a Test for Interfering Factors
   a. Verify test validity. All the negative control replicates should test negative; the sensitivity of the lysate reagent (λ) should be confirmed (i.e. the geometric mean endpoint of the standards must be between ½λ and 2λ) for the endotoxin in LRW (standard curve). If these conditions are not met, the test is invalid.

   b. If all replicates have endpoints at the same endotoxin concentration, that concentration is the result for the standards. If the endpoints are different for the replicate series, the geometric mean endpoint endotoxin concentration is determined as follows:

   \[
   GM = \text{antilog } \left( \frac{\Sigma e}{f} \right)
   \]

   where \( \Sigma e = \text{sum of log endpoint concentrations} \) and \( f = \text{number of replicate endpoints} \).

   c. The geometric mean endpoint of the test with the endotoxin diluted in sample should also be between ½λ and 2λ, if not the sample contains factors that interfere with the test and the test should be repeated using a greater dilution of the sample, not exceeding the MVD.

   d. MVD = maximum valid dilution is defined as the greatest dilution at which the endotoxin limit for the sample can be detected.

   \[
   \text{MVD} = \frac{\text{(Endotoxin limit) (Concentration of the sample)}}{\lambda}
   \]

   where \( \lambda \) is the sensitivity of the LAL reagent in EU/mL and the endotoxin limit is expressed in EU/unit of sample (e.g. EU/mg, EU/mEq or EU/mL) and the concentration is units of sample/mL. Endotoxin limits are given in pharmacopeia monographs or may be calculated (or verified) using the information in the USP BET chapter.

   e. Additionally, the geometric mean endpoint of each of the two tests must also be within twofold of each other otherwise the overall test is not valid. An example is shown below where the endpoint of the test using endotoxin dilutions in LRW is ½λ (valid) and the endpoint of the test using endotoxin dilutions in sample is 2λ (valid), the overall test is not valid because there is a fourfold difference between the two test results.

   \[
   \begin{array}{|c|c|c|c|c|}
   \hline
   \text{Endotoxin in LRW (Valid)} & \text{Endotoxin in Sample (Valid)} \\
   \hline
   2\lambda & \lambda & \frac{\lambda}{2} & \frac{\lambda}{4} & \text{Negative Control} \\
   + & + & + & - & - \\
   + & + & + & - & - \\
   \hline
   \end{array}
   \]

   The overall test is invalid since there is more than a twofold difference between the two endpoints, i.e. fourfold, 2λ – 1/2λ.