Pyrosate® CSE Quick Guide

Associates of Cape Cod, Inc.
*Your Endotoxin & Glucan Experts*

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.
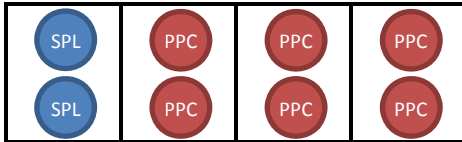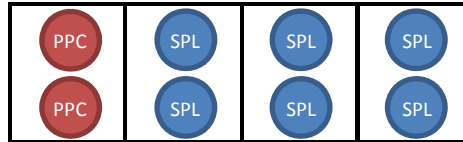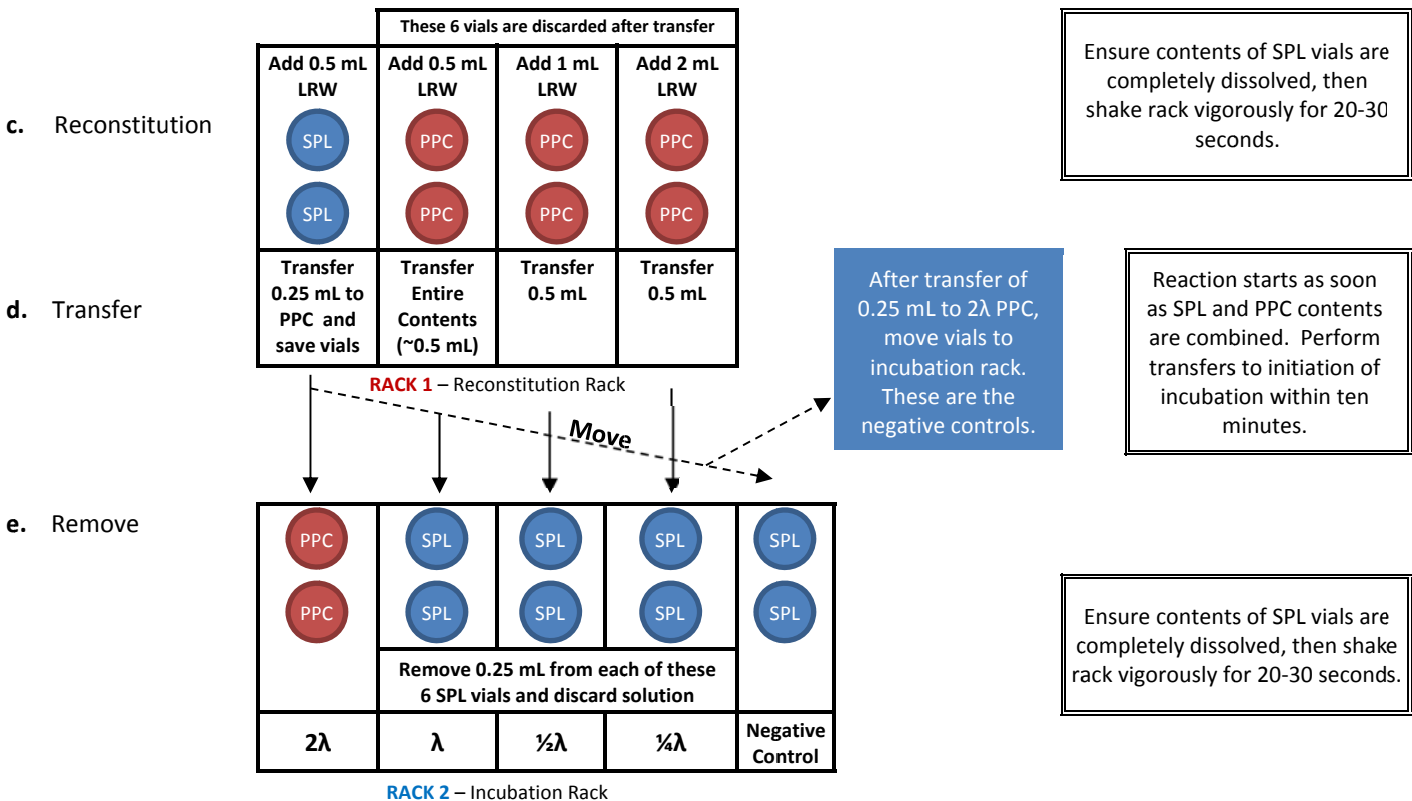

RACK 1 – Reconstitution Rack   **RACK 2** – Incubation Rack

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.

	These 6 vials are discarded after transfer		
Add 0.5 mL LRW	**Add 0.5 mL LRW**	**Add 1 mL LRW**	**Add 2 mL LRW**
SPL / SPL	PPC / PPC	PPC / PPC	PPC / PPC
Transfer 0.25 mL to PPC and save vials	**Transfer Entire Contents (~0.5 mL)**	**Transfer 0.5 mL**	**Transfer 0.5 mL**

c. **Reconstitution** — *Ensure contents of SPL vials are completely dissolved, then shake rack vigorously for 20-30 seconds.*

d. **Transfer**

RACK 1 – Reconstitution Rack

After transfer of 0.25 mL to 2λ PPC, move vials to incubation rack. These are the negative controls.

Reaction starts as soon as SPL and PPC contents are combined. Perform transfers to initiation of incubation within ten minutes.

Move

e. **Remove**

2λ	λ	½λ	¼λ	Negative Control
PPC / PPC	SPL / SPL	SPL / SPL	SPL / SPL	SPL / SPL
	Remove 0.25 mL from each of these 6 SPL vials and discard solution			

RACK 2 – Incubation Rack

Ensure contents of SPL vials are completely dissolved, then shake rack vigorously for 20-30 seconds.

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 ¼λ vials, etc.
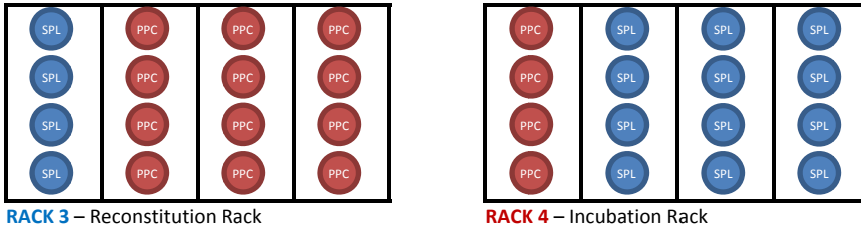
Positive test

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.

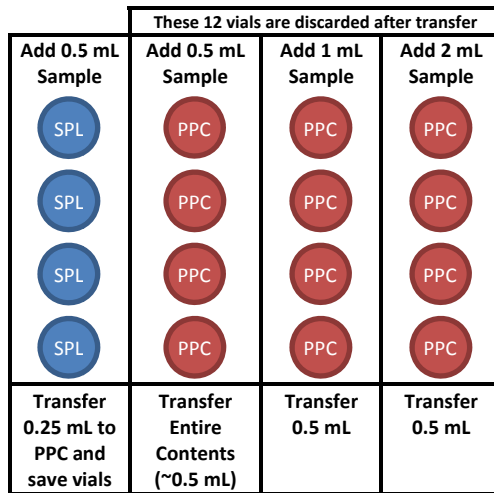**5. Set Up (Endotoxin diluted in sample)**

   a. Set up two racks of SPL and PPC vials in quadruplicate according to diagram below.

RACK 3 – Reconstitution Rack   RACK 4 – Incubation Rack

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.

|	These 12 vials are discarded after transfer		
Add 0.5 mL Sample	Add 0.5 mL Sample	Add 1 mL Sample	Add 2 mL Sample

d. Reconstitution

Ensure contents of SPL vials are completely dissolved, then shake rack vigorously for 20-30 seconds.

e. Transfer

Transfer 0.25 mL to PPC and save vials	Transfer Entire Contents (~0.5 mL)	Transfer 0.5 mL	Transfer 0.5 mL

RACK 3 – Reconstitution Rack

Move

After transfer of 0.25 mL to 2λ PPC, move vials to incubation rack. These are the negative controls.

Reaction starts as soon as SPL and PPC contents are combined. Perform transfers to initiation of incubation within ten minutes.

f. Remove
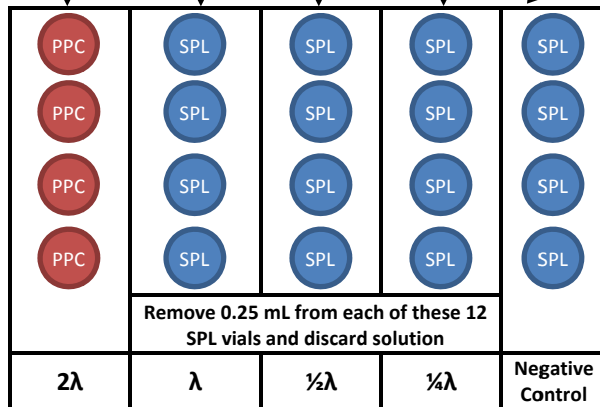
Remove 0.25 mL from each of these 12 SPL vials and discard solution

2λ	λ	½λ	¼λ	Negative Control

RACK 4 – Incubation Rack

Ensure contents of SPL vials are completely dissolved, then shake rack vigorously for 20-30 seconds.

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7. Testing

a. Incubate Rack 4 in a water bath equilibrated at $37 \pm 1°C$ 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.

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.

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:

$$GM = \text{antilog} \left(\sum e/f \right)$$

where $\sum e$ = sum of log endpoint concentrations and f = number of replicate endpoints.

c. The geometric mean endpoint of the test with the endotoxin diluted in sample should also be between ½λ and 2 λ, 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.

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

where λ 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.

Endotoxin in LRW (Valid)

2λ	λ	½λ	¼ λ	Negative Control
+	+	+	-	-
+	+	+	-	-

Endotoxin in Sample (Valid)

2λ	λ	½ λ	¼ λ	Negative Control
+	-	-	-	-
+	-	-	-	-
+	-	-	-	-
+	-	-	-	-

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

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