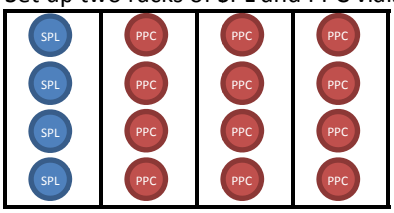
# Pyrosate® CSE Quick Guide

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

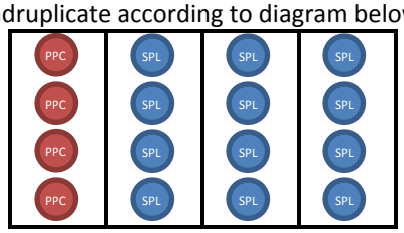
## Schematic for Confirmation of Labeled Lysate Sensitivity Using Pyrosate® PPC Vials

1. **The USP Bacterial Endotoxin Test chapter requires that Confirmation of Labeled Sensitivity be performed in quadruplicate.**

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



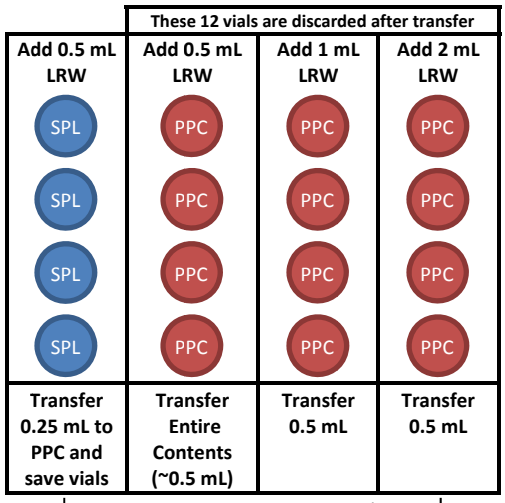

**RACK 1** – Reconstitution Rack




**RACK 2** – Incubation Rack

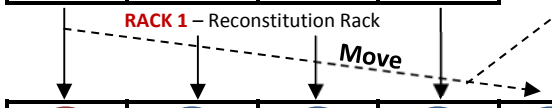
3. **Preparation**
   - **a.** Use a new pipette (or tip) for each transfer/removal combination.
   - **b.** Remove stoppers taking care not to contaminate the vials and test per instructions below.

**c.** Reconstitution




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

**d.** Transfer



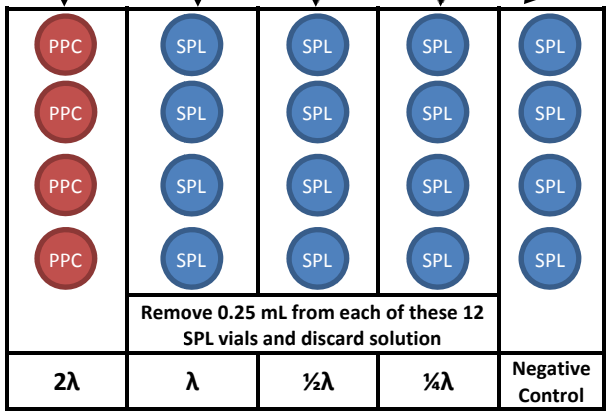

**RACK 1** – Reconstitution Rack




After transfer of 0.25mL 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.

**e.** Remove




**RACK 2** – Incubation Rack

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

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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. **Interpretation of Results of a Confirmation of Sensitivity**
   - **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 ½ λ and 2 λ ). 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:

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

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