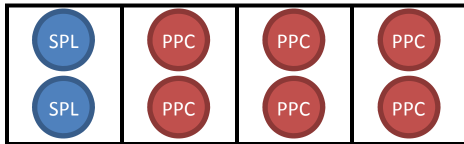
Pyrosate® CSE Quick Guide

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

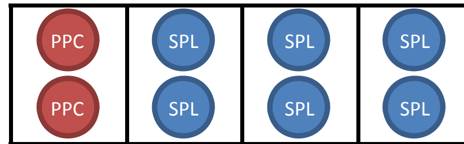
Schematic for Quantitative Test Using Pyrosate® PPC Vials

1. **The USP Bacterial Endotoxin Test chapter requires the Quantitative Test be performed in duplicate together with a standard curve test in duplicate.**

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



RACK 1 – Reconstitution Rack

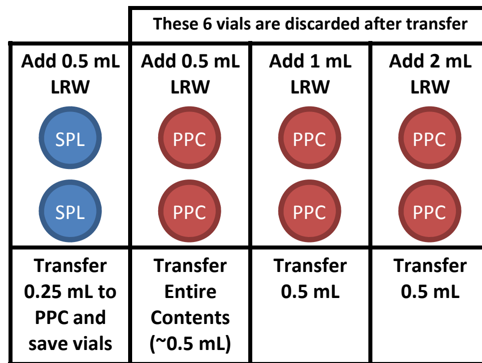


RACK 2 – Incubation Rack

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.

   c. Reconstitution

   d. Transfer



RACK 1 – Reconstitution Rack

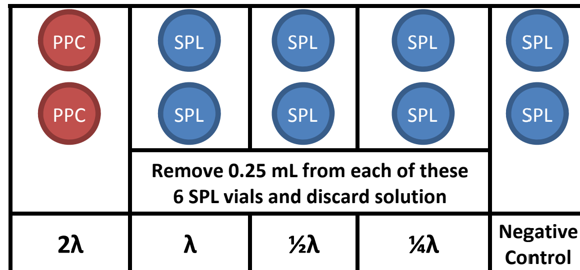
Ensure contents of SPL vials are completely dissolved, then shake rack vigorously for 20-30 seconds.

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



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.

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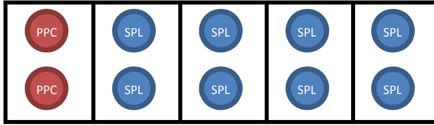
5. Set Up (Serial dilution of the sample)

a. Set up one rack of SPL and PPC in duplicate according to diagram below.



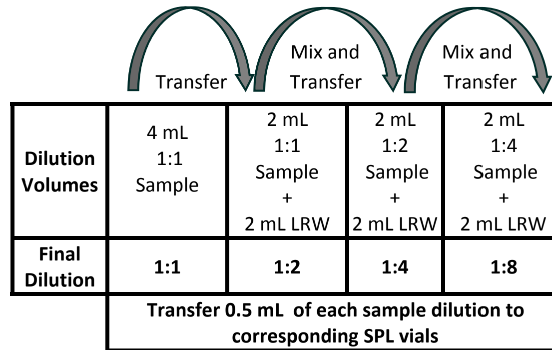
RACK 3 – Incubation Rack

6. Preparation

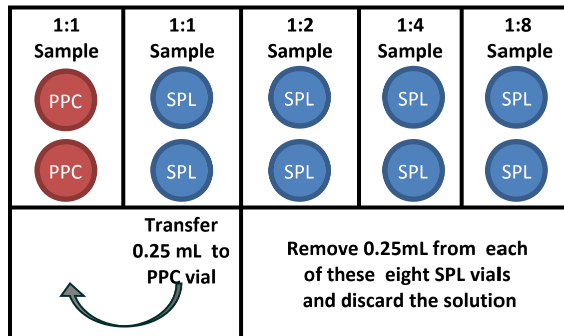
a. Use a new pipette (or tip) for each transfer/removal combination.

b. Remove stoppers from SPL and PPC vials just prior to reconstitution taking care not to contaminate the vials and test per instructions below.

c. Prepare at least three twofold dilutions of the test sample with LRW in dilution tubes (see below). The 1:1 undiluted sample corresponds to the selected dilution determined from the Test for Interfering Factors.



d. Reconstitution



RACK 3 – Incubation Rack

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

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

7. Testing

a. Incubate Rack 3 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.

8. Interpretation of Results of a Quantitative Test

a. Verify test validity: The negative controls should both 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 standard curve; both the PPCs in the sample test should also test positive. If these conditions are not met the test is invalid. 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 ∑e = sum of log endpoint concentrations and f = number of replicate endpoints.

b. For a valid test of a sample, if both replicates of all dilutions are negative, report the result as less than the concentration of λ (multiplied by the dilution factor of any initial dilution, if applicable).

c. For each of the replicates of the sample dilutions in which at least one of the concentrations of sample test positive, determine the endpoint dilution factor. (See section 2.2 of the package insert, Interpretation of Results of a Quantitative Test, for explanation of "endpoint dilution factor.")

d. Calculate the endotoxin concentration by multiplying the endpoint dilution factor by λ. Thus, if λ = 0.125 EU/mL and the endpoint dilution factor is 4, the result is 4 x 0.125 EU/mL, or 0.5 EU/mL. If both replicate endotoxin concentrations (calculated as described above) are the same, that concentration is the reportable result. If not, calculate and report the geometric mean of the two. If all dilutions of the sample test positive, either test additional dilutions to determine the endpoint or report the result as greater than or equal to λ multiplied by the highest dilution factor tested.

Note: If a dilution of the original sample was tested, then the result is multiplied by that dilution factor.

(See section 2.2 of the package insert, Interpretation of Results of a Quantitative Test for further details and examples of test interpretation).

Example of a test for a single sample using a lysate reagent sensitivity (λ) of 0.125 EU/mL:

Controls

2λ 0.25 EU/mL	**λ** 0.125 EU/mL	**½λ** 0.06 EU/mL	**¼λ** 0.03 EU/mL	**Negative control**
+	+	-	-	-
+	+	-	-	-

Sample

1x (undiluted)		**2x dilution**	**4x dilution**	**8x dilution**
PPC	**Sample**			
+	+	+	-	-
+	+	+	-	-

Interpretation
The test is valid because:
(1) the negative controls did not clot
(2) the endpoint concentration of the standard series is at λ (0.125 EU/mL), confirming the labeled sensitivity of the lysate reagent within a factor of two
(3) the PPCs clotted.

For both replicates of the sample the endpoint is at the twofold (2x) dilution (i.e. the endpoint dilution factor = 2). The endotoxin concentration of the sample is therefore 2 x λ or 0.25 EU/mL.