

**PSEUDOMONAS SPECIES
PRESUMPTIVE SPREAD PLATE METHOD
AND CONFIRMATION OF *PSEUDOMONAS AERUGINOSA***

PRINCIPLE

Presumptive *Pseudomonas aeruginosa* and total *Pseudomonas* bacteria are quantitated by a surface plating technique using a selective isolation medium. Caution must be exercised when applying the method since several species of *Pseudomonas* are opportunistic pathogens.

SCOPE

The method is applicable to the starches, syrups and sugars of the corn wet milling industry.

SPECIAL APPARATUS

Sterile Spreading Rods.

MEDIA AND REAGENTS

1. *Pseudomonas* Isolation Agar-PIA. Prepare medium according to manufacturer's directions. Add 20 mL glycerol per liter. Dispense in bottles and sterilize by autoclaving.
2. *Pseudomonas* Agar F-PAF. Prepare medium according to manufacturer's directions. Add 10 mL glycerol per liter. Sterilize by autoclaving. Dispense 15 mL of sterile medium into petri dishes, and solidify.
3. *Pseudomonas* Agar P-PAP. Prepare medium according to manufacturer's directions. Add 10 mL glycerol per liter. Sterilize by autoclaving. Dispense 15 mL of sterile media into petri dishes, and solidify.
4. Cetrimide Agar. Prepare medium according to manufacturer's directions. Add 10 mL glycerol per liter. Sterilize by autoclaving. Dispense 15 mL sterile media into petri dishes, and solidify.

**Microbiological Methods of the Member Companies of the
Corn Refiners Association**

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SPREAD PLATE METHOD — continued**

5. Glycerol
6. Butterfield's Phosphate Diluent

Stock solution: Dissolve 34 g of potassium dihydrogen phosphate (KH₂PO₄) in 500 mL of purified water, adjust to pH 7.2 with about 175 mL of 1N NaOH solution and dilute to 1 L volume. Store under refrigeration.

Diluent: Dilute 1.25 mL of stock solution of 1 L volume with purified water. Prepare dilution blanks using this solution.

7. Dilution Blanks

Fill dilution bottles to 80 mL or desired volume with Butterfield's Phosphate Diluent (Note 1).

PROCEDURE

- A. Quantitative Method for Presumptive Total *Pseudomonas*

Aseptically weigh 20 g of sample into a sterile 80 mL water blank and homogenize (Note 2). This is the primary dilution and represents a sample dilution factor of 5. Twenty mL of the primary dilution can be aseptically transferred to another 80 mL water blank, and the sample is diluted by a factor of 25. The number of dilutions depends on the individual sample and may be determined by past experience.

Pipet 0.5 mL (Note 3) of each sample dilution onto the surface of duplicate, dry PIA Agar plates (100 × 15 mm). Spread the inoculum evenly onto the surface of each plate with separate sterile glass spreading rods. Allow the surface of the plates to dry thoroughly. Invert the plates and incubate at 35-37°C for 48 hrs. (2 days).

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Count all pigmented and nonpigmented colonies on the plates. For the pyocyanin pigment producing species of *Pseudomonas aeruginosa*, the colonies will appear blue to blue green in color (Note 4).

- B. Quantitative Method for *Pseudomonas aeruginosa*
1. Follow the procedures above, and in place of *Pseudomonas* Isolation Agar use Cetrimide Agar. Count all pigmented and non-pigmented colonies on the plates. The colonies will range in color from blue, blue-green, yellow-green or non-pigmented.
 2. Pick two or more colonies from CEA and streak for isolation onto a plate of PAF and PAP agar. Incubate the plates at 35-37°C for 18-24 hours and examine the isolated colonies for pigment formation. Confirmed strains of *Pseudomonas aeruginosa* produce a fluorescent yellow and green pigment, which diffuses into PAF agar or a blue pigment in PAP. All non-typicals should be confirmed (Note 4).

CALCULATION

1. Total *Pseudomonas*:

Presumptive (+) *Pseudomonas* =
 count per gram of sample

$$\text{Average number of presumptive (+) colonies} \times \frac{\text{sample dilution factor}}{\text{mL of inoculum spread plated}}$$

2. Percent Confirmed *Pseudomonas aeruginosa*:

$$\text{Percent Confirmed } Pseudomonas \text{ aeruginosa} = \frac{\text{Number of Confirmed Colonies} \times 100}{\text{Total } Pseudomonas \text{ Count}}$$

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NOTES AND PRECAUTIONS

1. When sterilizing dilution blanks, a portion of the diluent may be lost. When this occurs, the sterilized blanks are brought to the proper volume with the sterile diluent.
2. When running counts on pregelatinized starches, no more than 5 g of sample per 95 mL of diluent may be used.
3. If high counts are anticipated, pipet 0.1 mL instead of 0.5 mL onto the plate.
4. To confirm presumptive (+) colonies as positive *Pseudomonas aeruginosa* or other species the appropriate morphological and biochemical tests must be performed on representative colonies by an experienced laboratory.

REFERENCE

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.