NSHS
National Seed Health System

VERSION: 1.0	**DATE:** 12/2012
PATHOGEN: Pseudomonas syringae pv. syringae	
HOST: common bean (Phaseolus vulgaris)	
COMMON NAME: bacterial brown spot	
METHOD: Be 2.1 Agar Plate Assay (Mohan and Schaad 1987)	
METHOD CLASS: TEMPORARY STANDARD (B)	
SAMPLE: 1 kg of seeds	

PROCEDURE:

1. Three one kg of seed are soaked in 3L of sterile saline solution with 0.01% Tween 20 for 20 hours at 5°C.

2. Each suspension is mixed with a sterile glass rod and a 150ml sample of the extract is drawn.

3. A 100ml potion of the extract is concentrated 10-fold by centrifugation at 12,000g for 10 min. The pellet is re-suspended in 10ml sterile saline (0.85% NaCl).

4. Aliquots of 0.1ml of 10-fold diluted, undiluted, and 10-fold concentrated extracts are plated onto selective media KBC and MSP in triplicate.

5. Plates are incubated at room temperature (23 ± 2°C) for 3 to 4 days.

6. The 10-fold concentrate is stored at 3-5°C until plates are read. This is re-centrifuged 10-fold more and plated as above if plates at the 10-fold concentrated sample only show a few saprophytic colonies.

7. Presumptive positive colonies are confirmed by biochemical and pathogenicity testing (Sands et al., 1980). Colonies on KBC are 3-3.5mm in diameter, flat, circular, translucent, creamy white, and showed blue fluorescence under UV light. Colonies on MSP are 3mm in diameter after 3 days at room temperature. They are circular, raised, globose, glistening, and light yellow with a less dense center. After 3 days the medium around the colony turned light yellow.

REFERENCES:

Mohan S. K. and Schaad N. W. 1987. An improved agar plating assay for detecting Pseudomonas syringae pv. syringae and P. s. pv. phaseolicola in contaminated bean seed. Phytopathology. 77(10):1390-1395 Sands D. C., Schroth, M. N. and Hildebrand, D. C. 1980. Pseudomonas. Pages 36-44 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. NW Schaad (ed.) American Phytopathological Society, St. Paul MN. 72p.