**VERSION:** 1.0  
**DATE:** 12/2012

**PATHOGEN:** Stemphylium botryosum f. sp. spinacia (sexual: Pleospora herbarum)  
**HOST:** spinach (Spinacia oleracea)  
**COMMON NAME:** leaf spot  
**METHOD:** Lcb 6.1 Freeze blotter method (Hernandez-Perez and du Toit, 2006)  
**METHOD CLASS:** STANDARD (A)  
**SAMPLE:** 400 seeds

**PROCEDURE:**

1. Place seed into a mesh tea strainer (Model 101, Venalicia Tea, Neuss, Germany).
2. Put the tea strainer in 150 ml of 1.2% NaOCl and shake manually for 60 seconds.
3. Triple-rinse seed in sterile deionized water and dry on a sterile paper towel in a laminar flow hood.
4. Flame-sterilize forceps, then plate 20 seed onto a sterile steel blue germination blotter (8.25 cm diameter; Anchor Paper Co., St. Paul, MN) that has been moistened with 4 ml of sterile deionized water in a 10-cm-diameter petri dish.
5. Seal the petri dishes with parafilm (Pechiney Plastic Packaging, Menasha, WI).
6. Incubate in the dark at 24°C for 25 h to imbibe moisture.
7. Freeze at −20°C for 22 to 25 h to prevent further germination.
8. Place in an incubator (Model I30BLL, Percival Scientific, Perry, IA) set at 24°C for 12 to 14 days with a 12h/12h day/night cycle, with cool-white fluorescent light and near-ultraviolet light by day.
9. Examine seeds approximately 4, 7, and 14 days after plating, at 8X to 100X magnification using a dissecting microscope for S. botryosum.

**REFERENCES:**