VERSION: 1.0	**DATE:** 5/2013
PATHOGEN: Ascochyta pinodes (sexual: Mycosphaerella pinodes)	
HOST: pea (Pisum sativum)	
COMMON NAME: blight	
METHOD: Pe 1.3 Blotter/agar (Gorfin, 2005)	
METHOD CLASS: TEMPORARY STANDARD (B)	
SAMPLE: 400 seeds	

PROCEDURE:

1. 400 seeds are surface sterilized with 1% sodium hypochlorite (NaOCl) solution for 5 minutes and then repeatedly rinsed with sterilized water.

2. Seeds are aseptically plated on Coon's Agar.

3. Incubate the seed sets for 12 to 20 days at 23±1°C and with alternating light and dark period of 12 hours. The light spectrum included near ultra-violate light (NUV) produced by black fluorescent lamp of 40W (Philips black fluorescent lamp).

4. Most of the fungi on seeds are slow growing under these conditions and observations are made after 20 days.

5. Fungi are identified by subsequent subculturing of newly growing hyphal tips on PDA.

Coon's Agar:

maltose	4 g
potassium nitrate	2 g
potassium hydrophosphate,	2.6 g

magnesium sulfate hydrated and	1.2 g
12g agar in one liter of distilled water (Bretag et al., 1995).	

REFERENCES:

Bretag, T. W., Price, T. V. and Keane, P. J. 1995. Importance of seed-borne inoculum in the etiology of the Ascochyta blight complex of field pea (Pisum sativum L.) grown in Victoria. Australian Journal Experimental Agriculture. 35:525–530.

Gorfu, D. and Sangchote, S. 2005. Fungi associated with field pea seeds from Ethiopia and seed transmission of Ascochyta pinodes. Seed Sci. & Technol. 33:387-396.