DATE:

Name:

THE TOPIC: **Culture Indexing for Bacterial and Fungal Contaminants**

The presence of latent microbial contaminants in tissue cultures affects growth and development, multiplication rate and acclimatization. Indexing is usually accomplished by inoculating segments or intact shoots into selection medium. For indexing of latent bacterial contaminants of tissue cultures is recommended a broad-spectrum Leifert and Waites Sterility Test Medium (Leifert *et al.* 1989) or B523 (Viss *et al*. 1991).

MATERIAL: Nodal segments of potato, carnation, raspberry and tobacco long-term cultures

MEDIA:

Agar solidified Leifert and Waites Medium (M-S basal salts medium 2,2g.l-1, Meat extract 7g.l^{-1}, Glucose 5g.l^{-1}, Peptone 4 g.l^{-1}, $NaCl_2$ 2 g.l^{-1}, Sucrose 15 g.l^{-1}, Yeast Extract 10 g.$l^{-1)}$

Agar solidified B523 medium (Caseinhydrolyzate 8 g.l^{-1}, $MgSO_4$. 7 H_2O 0,15 g.l^{-1}, K_2HPO_4 2 g.l^{-1}, Yeast Extract 4 g.l^{-1}, Sucrose 10 g.l^{-1}, agar 10 g.l^{-1})

Maintenance M-S medium (Murashige and Skoog)

PROCEDURE:

1. Remove shoots from culture and place in a sterile Petri dish in a laminar flow hood.
2. Cut the shoots into single node segments or thin cross-sectional disks leaving the shoot tips intact.
3. Transfer the shoot tips onto maintenance medium.
4. Inoculate the B523 agar medium by digging the segment into the medium, slowly lift out of the medium and place onto the medium. This procedure inoculates lower oxygen levels of the medium that may promote the growth of some microbes. Do the same type of inoculation to M-S medium.
5. Label corresponding segments, disks and shoot tips cultures.
6. Incubate inoculated Petri dishes in the dark at 22-30˚C for 3 weeks.

EVALUATION

Screen the cultures for visible contamination in the following period of cultivation.

Clouding of the liquid media or colonies on the surface of the medium and/or halo in the medium indicate the presence of cultivable contaminants in the segments or disks. The contaminated cultures should be autoclaved before disposing.

Compare contamination of the corresponding segments, disks and shoot tips cultures cultivated on both agar media.

LITERATURE:

Leifert *et al.* (1989): J. Appl. Bacter. 67: 351 – 361.

Viss *et al*. (1991): In Vitro Cell Dev. Biol.-Pl. 27P, 42.