APPENDIX II

CORNMEAL TWEEN-80/OXGALL AGAR

I. Purpose

To be used for yeast morphology when the germ tube is negative, but further identification is required as outlined in the section: Isolation and Identification. Cornmeal Tween-80 provides excellent diagnostic morphological features for yeast identification, but produces chlamydospores more slowly than oxgall agar.

II. Procedure

A. Cornmeal Tween 80:

1. Using a sterile wire loop, inoculate a small portion of a yeast colony by making two parallel streaks a few mm apart on the surface of a cornmeal agar plate. Do not cut into the agar.

2. Streak over the lines in a "zigzag" fashion (Dolmau technique).

3. Place a clean coverslip over the streaked area and press gently.

4. Include controls *T. glabrata* (negative) and *C. parapsilosis* (positive).

5. Incubate at 25-28^{0}C for 48 hours.

6. Using the light microscope examine under low power and high dry objectives for the presence of hyphae, pseudohyphae, blastoconidia, chlamydospore and/ or arthroconidia. The plate may need to be reincubated if the morphology is not fully developed (eg. arthroconidia formation).

B. Oxgall:

Oxgall agar is specifically used to show chlamydospores. Oxgall does not always give the classic diagnostic morphology of yeast as is seen with the Cornmeal Tween-80 agar.

1. Using a sterile spade shaped spatula, inoculate lightly by making 2 to 3 parallel cuts approximately 1/2 inch apart at 45^0 angle. Avoid cutting the agar through to the bottom.

2. Apply a clean coverslip on the inoculated area and press gently.

3. Include controls *C. albicans* (positive) and *C. tropicalis* (negative).

4. Incubate at 28^0C for up to 48 hours.

5. Examine the areas where the agar is cut under low and high dry objectives using the light microscope.

6. Observe for the presence of hyphae, pseudohyphae, blastoconidia, chlamydospores and arthroconidia.

7. Refer to Table 1. "Identification of yeast" in the section, Isolation and Identification for interpretation.

   Note: Yeast producing chlamydospores on cornmeal and/ or oxgall are reported as *Candida albicans*. Yeast not producing chlamydospores or pseudohyphae may require further testing.

III. References

1. J.B. Fisher, Julius Kane, "Production of Chlamydospores by *Candida albicans* cultivated on dilute Oxgall Agar". Mycopath. 35:223-229, 1968.