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Primary Isolation Medium for *Cryptococcus neoformans*

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The isolation and identification of *Cryptococcus neoformans* is improved by use of a potato dextrose medium containing urea-antibiotic supplements and a *p*H of 3.5.

The isolation of *Cryptococcus neoformans* from contaminated clinical specimens is rarely successful in laboratories other than those having personnel with special competence in mycologic techniques. Difficulties associated with recognition of the organism in culture and the lack of a suitable medium for isolation of the fungus have comprised formidable obstacles. The recent interest accorded this organism, both as a primary cause of pulmonary disease (1, 4) and as a secondary invader associated with certain malignant lesions (2), has given further impetus to isolation and identification techniques. Shields and Ajello (3) recently introduced a selective medium containing glucose, creatinine, and an extract of *Guizotia abyssinica* (thistle seed) on which colonies of *C. neoformans* produce a brown pigment; other species of *Cryptococcus* and *Candida* grow on this medium without producing pigment. A new medium, developed to facilitate the primary isolation of *C. neoformans*, is herewith described. The basal medium is comprised of commercial potato dextrose agar. To 1 liter of this base, while still molten, 100 ml of filter-sterilized commercial urea agar base is added. The *p*H is adjusted to 3.5 by the addition of approximately 35 ml of 0.5 N HCl to 1,000 ml of the medium. Before dispensing into petri dishes, sufficient chloramphenicol is added to provide a concentration of 100 units/ml. The medium is bright yellow in color as a result of the reaction at *p*H 3.5 of the phenol red indicator in the urea agar base.

Clinical specimens can be plated directly onto the agar or after appropriate digestion has been carried out. It is recommended that when sputum digestion methods such as those employing *N*-acetyl-L-cysteine or Cleland's reagent are used, the amount of sodium hydroxide be kept to a minimum, since the purpose of the digestion here is to liquefy the sputum and not to inhibit bacterial growth. After digestion, the *p*H of the mixture is rendered acidic with dilute HCl in order to preserve the yellow reaction of the medium.

Growth on the medium is restricted to *Candida* and *Cryptococcus* species. Saprophytic molds can be inhibited, if necessary, by the addition of diphenyl ($C_6H_5C_6H_5$) to a concentration of 0.01% as described by Shields and Ajello (3). Growth of *Cryptococcus* species is recognized as a moist, white colony surrounded by a red halo, which results from the breakdown of urea. *Candida* species will grow on the agar, but without a color change in the medium. Incubation of cultures at 37 C eliminates the growth of saprophytic cryptococci, thus making the medium selective for pathogenic cryptococci.

In preliminary experiments with this modified urea-agar, growth of stock cultures of *Proteus*, *Staphylococcus aureus*, and *Escherichia coli* was completely inhibited at *p*H 3.5. Values above and below this figure were unsatisfactory. Effective recovery of the fungus was made from sputum specimens inoculated with *C. neoformans* to simulate actual clinical conditions. Sputum digestants were nontoxic to the fungus when the *p*H during digestion was approximately 8.

In a brief field trial with the medium at the Birmingham Veterans Administration Hospital, K. Mason (*personal communication*) demonstrated a similar rate of recovery of *C. neoformans* from patients with pulmonary cryptococcal infection on the urea-agar and the medium of Shields and Ajello (3).

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