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Modified India Ink Preparation for *Cryptococcus neoformans* in Cerebrospinal Fluid Specimens

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A novel modified India ink technique for the diagnosis of *Cryptococcus neoformans* in cerebrospinal fluid specimens is described. It employs 2% chromium mercury and India ink. This technique allows a clear identification of some external and internal structures of the organism. Three layers from the outer capsule that have previously been discerned only by electron microscopy are distinguished.

Cryptococcus neoformans is an opportunistic yeast species that may cause meningitis, particularly in immunocompromised patients (3). Presumptive laboratory diagnosis is often based on the observation of the characteristic mucopolysaccharide capsule by means of the India ink preparation method (1, 5), and the presence of *C. neoformans* is confirmed by culture. We report here a novel technique which allows a better distinction of the organism.

Ten specimens of cerebrospinal fluid (CSF) that were suspected for *C. neoformans* meningitis and that corresponded to samples from adult human immunodeficiency virus-positive patients were received for further microbiologic study.

With these specimens, we compared the conventional India ink preparation method with a modified preparation technique developed by us. That all the specimens contained *C. neoformans* was confirmed by culture with Sabouraud's dextrose agar and brain heart infusion agar (2).

The modified technique employs 2% chromium mercury. A small drop of CSF is placed on a clean glass slide. Then, a small drop of 2% chromium mercury is mixed with the CSF on the slide. Immediately, a small amount of India ink (Pelikan Drawing Ink) is added. Finally, the coverslip is mounted and the preparation is observed with a bright-field microscope at magnifications of ×100, ×400, and ×1,000.

By this technique, we observed some external and internal structures of the yeast cells with ×40 and ×100 objectives. Thus, three layers from the outer capsule that have previously been discerned only by electron microscopy were distinguished, namely, the lucent stratum of the capsule, the fibrillar material of the capsule, and the light zone (4). In addition, internal corpuscles corresponding to different sizes of endogenous spores of the organism were also clearly distinguished (Fig. 1, *see following page*). The conventional India ink preparation showed the yeast cells with their usual characteristics (data not shown). This novel preparation mimics a polychromatic preparation, even though no color stains were used during the procedure. This seemingly polychromatic presentation of *C. neoformans* allowed the distinction of microscopic air bubbles that sometimes are mistaken for *C. neoformans* when the conventional India ink preparation method is used.

We propose this simple, inexpensive, and reliable preparation for the assessment of CSF specimens suspected of containing *C. neoformans*.

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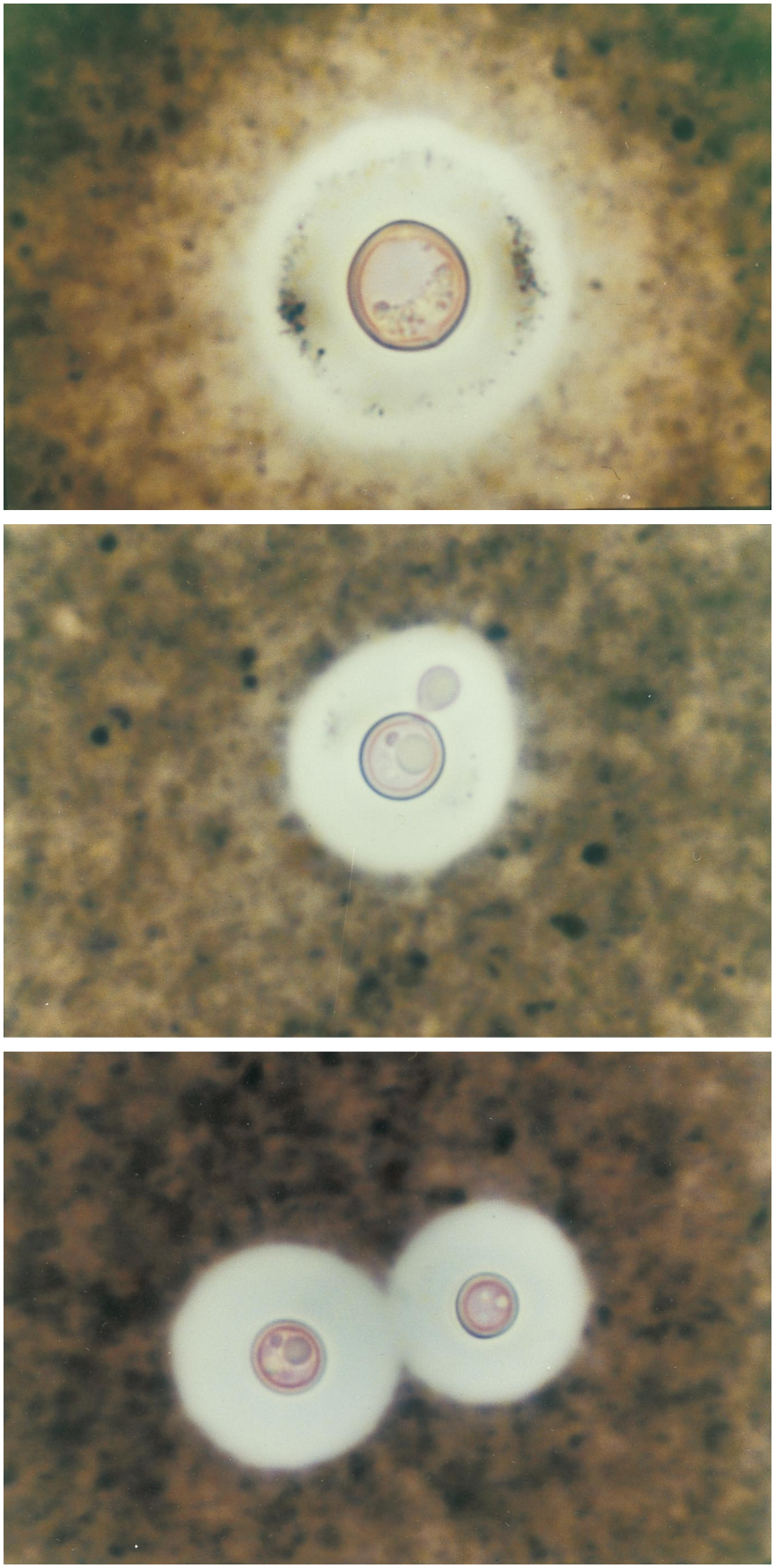

FIG. 1. The external and internal structures of *C. neoformans* are shown by means of a modified India ink preparation. Magnification, ca. ×1,000.