NOTES

Detection of Fungi in Clinical Specimens by Phase-Contrast Microscopy

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During 1973 and 1974, the following fungi were detected in clinical specimens by using phase-contrast microscopy: *Blastomyces dermatitidis*, 5; *Coccidioides immitis*, 3; *Cryptococcus neoformans*, 11; other yeasts 918; dermatophytes, 863; *Mucor* species, 1; and *Aspergillus fumigatus*, 16. This technique allows rapid detection and, in many instances, immediate identification of fungi in clinical specimens.

The laboratory diagnosis of mycotic infection is based on definitive identification of the etiological agent. In most instances this requires 2 to 3 weeks, the period needed for characteristic morphological features to develop in culture; for the dimorphic fungi, an additional 2 to 3 weeks is needed for conversion of the filamentous form to the tissue form.

In many instances, direct microscopic examination of the specimen as soon as it reaches the laboratory can provide a tentative diagnosis or, in some cases, a definitive diagnosis (1). Most reports have dealt with the india ink preparation for recognition of *Cryptococcus neoformans* in cerebrospinal fluid; however, sputum cytological examinations have been reported to be helpful in detecting fungi in a few instances (2, 3). This paper presents observations made using phase-contrast microscopy and describes the characteristics of those organisms that can be recognized by this method.

In the clinical mycology laboratory at the Mayo Clinic, all urine specimens, skin scrapings, transtracheal aspirates, bronchial washings, and other normally sterile body fluids are examined routinely by phase-contrast microscopy. The clinicians are aware that this technique is available and frequently order such examination on sputum, corneal scrapings, pus from skin lesions, and throat swabs.

All specimens except urine are mixed, on a slide, with 10% potassium hydroxide containing 10% glycerin, covered with a no. 1 glass cover slip, and placed in a moist chamber for 15 to 30 min. Urine specimens are centrifuged and a drop of the sediment is placed on a slide and

covered with a no. 1 glass cover slip. With specimens containing a large amount of cellular debris, the slide is gently passed through a flame to hasten clearing of the material and gentle pressure is applied to the cover slip to disperse the material.

All specimens are examined under a Leitz phase-contrast microscope with a $\times 40$ Phaco objective. When certain morphological characteristics are found, the physician is notified of the preliminary or final identification of the organism.

During the past few years, direct examina-

FIG. 1. *Blastomyces dermatitidis* in sputum. Characteristic yeast form has budding cell attached by broad base (arrow). Also note "double contoured" appearance of cell wall. ($\times 2,000$.)

TABLE 1. *Organisms observed by phase-contrast microscopy of specimens in 1973 and 1974*

Organism	No.	Specimen type	Identification
<i>Blastomyces dermatitidis</i>	5	Sputum; pus; transtracheal aspirate	Definitive
<i>Coccidioides immitis</i>	3	Sputum; pus	Definitive
<i>Cryptococcus neoformans</i>	11	Sputum; bone; cerebrospinal fluid	Definitive (to genus)
Dermatophytes ^b	863	Skin; nails	Tentative
Other yeasts ^a	918	Urine; sputum; skin; throat swab; catheter tip	Tentative
Phycomycete (zygomycete) ^c	1	Pus from skin wound	Definitive
<i>Aspergillus</i> sp. ^d	16	Sputum; transtracheal aspirate	Tentative

^a Includes *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, and *Torulopsis glabrata*.

^b Includes the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*.

^c Genera most frequently encountered are *Mucor*, *Rhizopus*, *Absidia* and *Mortierella*.

^d *A. fumigatus* is the most frequently encountered species but other species of aspergilli as well as other genera of fungi may exhibit the same features.

FIG. 2. *Coccidioides immitis* in sputum. Large thick-walled spherules with few endospores scattered within interior of spherule (a) or cleavage furrows developing along periphery to form endospores (b). ($\times 2,000$.)

tion of specimens by phase-contrast microscopy has allowed our laboratory to make tentative diagnoses of dermatophytosis, candidosis, phycomycosis, and aspergillosis. All of these were

FIG. 3. *Cryptococcus neoformans* in sputum. Spherical yeast cell is surrounded by large capsule with small bud arising from parent cell. ($\times 2,000$.)

confirmed by culture. Definitive diagnoses of cryptococcosis, blastomycosis, and coccidioidomycosis were made within minutes after the specimen reached the laboratory. Table 1 lists the organisms and types of specimens in which they were observed. Figures 1 through 7 show the appearance of the organisms by phase-contrast microscopy. A brief description of the morphological characteristics is presented in each legend.

Bright-field microscopy is satisfactory for the direct examination of specimens if the light intensity is decreased; however, phase-contrast microscopy is better because structures are more clearly delineated without loss of light.

It is hoped that clinical microbiology laborato-

FIG. 4. *Dermatophyte* in skin scraping. Septate hyphae intertwine among squamous cells. ($\times 2,000$.)

FIG. 5. *Candida albicans* in urine. Hyphae (a) and budding yeasts (b) appear among epithelial cells. ($\times 2,000$.)

FIG. 6. *Mucor* species in pus from skin lesion. The large, branching, ribbonlike aseptate hyphae are indicative of a phycomycete. ($\times 2,400$.)

FIG. 7. *Aspergillus fumigatus* in sputum. The septate hyphae show dichotomous branching (arrows). ($\times 2,000$).

ries will take advantage of this simple technique to provide a rapid, tentative, and often definitive, identification of fungi that cause infection in man.

LITERATURE CITED

1. Kurung, J. M. 1957. The examination of sputum. III. Search for fungal spores. *Am. Rev. Tuberc. Pulm. Dis.* 76:679-682.
2. Prolla, J. C., U. W. Rosa, and R. G. Xavier. 1970. The detection of *Cryptococcus neoformans* in sputum cytology: report of one case. *Acta Cytol.* 14:87-91.
3. Sutliff, W. D., and T. P. Cruthirds. 1973. *Blastomyces dermatitidis* in cytologic preparations. *Am. Rev. Respir. Dis.* 108:149-151.