

January 2006

0601-1: simulated CSF: *Cryptococcus neoformans*

CMPT QA: Internal validation demonstrated pure growth of *C. neoformans*; confirmed by the Reference Laboratory.

RESULTS: All participants correctly identified the organism as *C. neoformans*. As listed in Table 1, laboratories reported a variety of media and methods to isolate and identify this fungus.

Media and Methods reported	No. labs
IMA, SAB, BHI, 30 C, Cornmeal agar, India ink, API 20C Aux, Urease	1
SAB, BHI, 30 C, Auxacolor, India Ink	1
IMA, BHIA, BHI with Fildes, 28 C, CLA, Urea	1
PDA, BAP, BSGGYA, 30 C, TOC, Urease, Axacolor 2	1
IMA, BHIA, 30 C, Cornmeal, India Ink, API 20C Aux	1
IMA 25C, 37C, CLA test	1
SABHI with blood, Littman, Mycosel 25 C, Urease, Uni-Yeast, API 20 C	1
IMA, Mycosel, SDA, 25 C	1
IMA, BHI, 30 C	2

Key: IMA, inhibitory mold agar; BHI, brain heart infusion; CLA, cryptococcal latex agglutination; PDA, Potato Dextrose Agar; SDA, Sabouraud's Dextrose Agar; DTM, Dermatophyte Test Medium; BCPCYA, Bromcresol Purple Casein Yeast Extract agar; LOM, Litman Oxgall Medium; TOC, Tween 80, Oxgall, Caffeic Acid agar; BSGGYA, Bismuth sulfite glucose glycine yeast agar

CLINICAL SIGNIFICANCE^{1,2} Patients with central nervous system (CNS) *Cryptococcosis* may be either asymptomatic or present with insidious symptoms such as mild headaches, memory lapses or personality changes or have overt signs of meningitis, sometimes with localising signs, e.g., acute onset blindness. Many of the symptoms are similar to those seen in other diseases and include: fever, fatigue, stiff neck, body aches, headaches (often severe), nausea/vomiting, and skin lesions. Classic symptoms are not evident in infants and are often not seen in the elderly.

People with compromised immune systems, particularly HIV-positive people with T-cell counts below 50, are more likely to experience cryptococcal meningitis. Cryptococcal meningitis is recognized as one of the AIDS defining conditions. It acts as a diagnostic marker for further investigations to establish the retroviral infection. Cryptococcosis also occurs in patients, who are not infected with human immunodeficiency virus (HIV), such as those who are on prolonged high dose or therapeutic dose corticosteroid therapy, patients with lymphoreticular malignancies as well as sarcoidosis even in the absence of

corticosteroid therapy. Up to 30% of cases occur in immunocompetent patients. If not treated correctly, cryptococcal meningitis can be fatal. Pulmonary infections are common, but *Cryptococcus neoformans* can also cause disease less commonly in the kidneys, skin, urinary tract, lymph nodes, and rarely the GI tract.

ECOLOGY¹ There are 19 species in the genus *Cryptococcus*, but *C. neoformans* is considered the only human pathogen. There are four serotypes, A, B, C, and D, based on capsular epitopes. Serotypes A, D, and AD conform to *C. neoformans* var. *grubeei*, and serotypes B and C conform to *C. neoformans* var. *gattii*. Serotype A is worldwide and is responsible for most of the infections in AIDS patients. Serotype C is mostly in Southern California, Serotype B in Vancouver Island, Australia, Hawaii, S. California, Africa, and Southeast Asia. Most isolates of serotype D are recovered in Europe. The source for serotypes A and D is bat guano and pigeon dung. The ecology for serotypes B and C is quite different. Various species of gum and eucalyptus trees are thought to be reservoirs in tropical countries, and several types of conifers (Coastal Douglas fir) and deciduous trees on Vancouver Island. The four serotypes differ from each other genetically, epidemiologically, ecologically, and biochemically. Standard laboratory tests do not differentiate between the serotypes. There is media available to distinguish serotypes A and D from B and C but these are not available commercially. Clinical isolates should be referred to a reference laboratory for serotyping.

Breathing in the spores of the *Cryptococcus* fungus found in the soil and on trees causes the infection and not from contact with an infected person or animal. *Cryptococcus* is unique in that the yeast form is the infective form and is the form found in the tissues. In the Vancouver Island cases the incubation period was estimated to be between 2 to 9 months after exposure³.

ISOLATION^{4,5} *Cryptococcus neoformans* is readily grown on common mycological and bacteriological media without cycloheximide such as brain-heart infusion broth, Sabouraud dextrose, blood, and chocolate agars. Any nonpigmented, round, encapsulated yeast recovered from spinal fluid should be considered to be presumptive *C. neoformans* until confirmed.

IDENTIFICATION Microscopic morphology
India Ink facilitates the visualization of cryptococcal polysaccharide capsules. The capsular material of cryptococci displaces the colloidal

carbon particles of the ink so that the capsule appears as a clear halo around the microorganisms against a black background. All fields need to be searched carefully as few organisms may be present. India ink preparations show single budding, oval to round cells (4 to 10 μm in diameter) with thin dark walls and narrow necks between mother and daughter cells. Buds detach easily and organisms observed directly in tissue and from cultures often demonstrate no buds. Rare strains may have multiple buds and pseudohyphae. Refractile, wide capsules may be demonstrated, especially in preparations made from the specimen and may not be apparent in wet mounts made from cultures. However, the size of the capsule is variable depending on the clinical specimen, stain used, growth media and host factors. **Calcofluor white or 20% KOH** may also be used in direct clinical material such as tissue or CSF. The sensitivity using India ink is estimated to be only 43%; so negative samples should be further tested with a backup method. Cryptococcus may be seen on **Gram** smears of CSF.

Characteristic morphology of *C. neoformans* does not pose any difficulty in recognizing the fungus. But unusual forms may be produced in clinical material that can give rise to diagnostic dilemma. For example, in one reported case of primary cryptococcosis of lungs, pleural fluid yielded giant cells of *C. neoformans* with thick capsules averaging over 40 μm ⁷. In contrast, *C. neoformans* recovered from AIDS patients has been reported to have smaller capsules, although this trait is not consistently observed. A rare morphology of “hand mirror” appearance in direct microscopic examination of both CSF and sputum from a patient with AIDS has also been reported.

Cryptococcal Antigen Tests Serodiagnostic tests for the detection of cryptococcal antigen are easily performed in the clinical laboratory or should be referred out to a reference laboratory when cryptococcal meningitis is suspected. This test is considered more sensitive than Gram smear or culture of CSF; though rare, false-positive and false-negative results have occurred. One explanation for false-negative results is the host response inhibits the capsule development needed to produce a detectable antigen response.

Colony morphology After 48 to 72 hours (usually within 7 days) shiny or opaque, moist to mucoid to runny, non-pigmented/cream-colored colonies appear. *Cryptococcus neoformans* usually grows equally well at 25°C and 37°C, whereas some of the other species of this genus will not grow well, if at all, at 37°C. Capsule production is enhanced on media at 37°C, increased CO₂, addition of 1% glucose or passaging the organism in broth with added serum.

In some cases, growth has taken as long as 10 days after start of incubation. Negative microscopic findings of the initial sample, likely missed due to the small numbers of organisms present, may yield positive culture results.

Classical Tests Confirmation to species level may be accomplished through evidence of growth at 37°C, positive rapid urease test, negative germ tube test, and production of melanin due to the enzyme laccase demonstrated by a rapid positive caffeic acid disc test or on bird-seed agar. Note that some other yeasts produce urease and highly mucoid colonies of *C. neoformans* may not produce enough melanin to be clearly positive¹. Isolates can be readily identified to species level by several commercial systems.

TREATMENT³ IV amphotericin B, with or without a second agent such as 5-FC in selected cases, is recommended for cases of meningitis. Step-down to oral fluconazole is monitored closely. Total treatment for these cases may be up to six months. There is no vaccine presently to prevent disease, but research on an animal vaccine shows promise that one will be developed for animals and humans.

REFERENCES

1. Perfect JR. 2005. *Cryptococcus neoformans*. Chapter 261. In GL Mandell, JE Bennett, R Dolin (eds.) *Mandell, Douglas and Bennett's Principles and practice of infectious diseases*. 6th ed., Churchill Livingstone, Philadelphia, Pa.
2. Mitchell TG, Perfect JR. 1995. Cryptococcosis in the Era of AIDS—100 Years after the Discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev.* 8:4 p. 515-548.
3. Kibsey P. 2003. Update on *Cryptococcus neoformans vargattii* outbreak on Vancouver Island 1999-2003. *CMPT Connections:* 7:3 Fall p. 2-3.
4. CMPT critique M013-5 CSF: *C. neoformans*, Nov. 2001.
5. CMPT critique M034-4 CSF: *C. neoformans*. Feb. 2004.
6. Hazen KC, Howell SA. *Candida, Cryptococcus* and other yeasts of medical importance In PR Murray PR et al. (eds.) *Manual of Clinical Microbiology*. 8th ed. Vol. 2. Ch. 119. ASM Press, Washington, DC.
7. Shashikala , Kanungo R, Srinivasan S, Mathew R, Kannan M. Unusual morphological forms of *cryptococcus neoformans* in cerebrospinal fluid. *Indian J Med Microbiol* [serial online] 2004 [cited 2006 Apr 3];22:188-190. Available from: <http://www.ijmm.org/article.asp?issn=0255-0857;year=2004;volume=22;issue=3;spage=188;epage=190;aualast=Shashikala>