Safety Precautions for Working with Cryptococcus neoformans

The basidiomycete fungus Cryptococcus neoformans is an invasive opportunistic pathogen of the central nervous system and the most frequent cause of fungal meningitis worldwide. Although Cryptococcus is a problem in the United States, it is significantly more prevalent and especially devastating in the developing world, such as sub-Saharan Africa, resulting in in more than 625,000 deaths per year worldwide.

C. neoformans survives in the environment within soil, trees, and bird guano, where it can interact with wild animals or microbial predators, maintaining its virulence. Human infection is thought to be acquired by inhalation of desiccated yeast cells or spores from an environmental source. C. neoformans can colonize the host respiratory tract without producing any disease. Infection is typically asymptomatic, and it can be either cleared or enter a dormant, latent form. When host immunity is compromised, the dormant form can be reactivated and disseminate hematogenously to cause systemic infection. C. neoformans can infect or spread to any organ to cause localized infections involving the skin, eyes, myocardium, bones, joints, lungs, prostate gland, or urinary tract, in addition to its propensity to infect the central nervous systems.

The following diseases and medications are risk factors for C. neoformans infection and are associated with at least some degree of immunosuppression:

- HIV/AIDs (CD4 < 100cells/mm)
- Corticosteroids or other immunosuppressive medications for cancer, chemotherapy, or organ transplants
- Solid organ transplantation
- Diabetes mellitus
- Heart, lung, or liver disease
- Pregnancy

Even otherwise healthy, fully immunocompetent individuals can develop cryptococcosis, as may well be the case in a lab accident. We therefore take the following precautions for working with C. neoformans:

- Individuals who are immunosuppressed, HIV-positive, pregnant, planning a pregnancy should not work with live C. neoformans.
- Live C. neoformans should always be treated with respect. Laboratory acquired infections may result from inhalation, cuts, or bites from infected lab mice. Exercise extreme care whenever handling spores or using sharps (needles, forceps, Pasteur pipettes, etc.). Also note that hemocytometer coverslips may have sharp edges! Special blunt needles are available for routine needle passage of infected cells. Sharp needles should only rarely by required (e.g. for animal inoculation).
- All infected material must be decontaminated (in bleach or by autoclaving) immediately after use. Please pay particular attention to sharps and hemocytometer cover glasses.

Any possible infection, no matter how small or unlikely, must be reported immediately and should be treated as outlined below. The incubation period is unknown as C. neoformans can colonize in the host respiratory tract for months to years without causing any clinical symptoms.
Signs and symptoms of cryptococcosis include: (1) pneumonia-like illness (shortness of breath, cough and fever), (2) meningitis (persistent headache, nausea, dizziness, impaired memory and judgment), and (3) skin lesions which commonly begin painlessly and have a variety of presentations. Mortality of symptomatic disease in developed nations is around 12%.

**In Case of Possible Laboratory Infection with Cryptococcus neoformans**

**What to do:**

- First of all, don’t panic.
- Immediate Action by Route of Exposure
  - Needle stick, Animal Bite or Laceration: Wash the area with soap and running water.
  - Mucous membranes (eye, nose, mouth): If contaminated material is splashed or sprayed contaminating the eyes, nose or mouth: Flush the eyes for 10-15 minutes. Rinse mouth out with clean water and do not swallow.
  - Inhalation: If contaminated materials are aerosolized outside of primary containment and potentially inhaled, rinse mouth twice expelling the rinsate. Do not swallow.
- Try to clarify the nature of your exposure:
  - Needle-stick, broken pipette, glass cut, eye-splash, other?
  - Known/likely/unlikely to be contaminated with C. neoformans
  - What strain: The lab routinely works with and cultures the wild type strain H99 (Serotype A) and mutants derived from, which is responsible for about 95% of cryptococcosis cases worldwide and is implicated in the majority of the AIDS co-infections.
  - Any transgenic plasmids, markers, reporters?
  - Drug-resistance status?
- Contact Kerry Smith immediately at 864-247-7905 (cell) any time day/night
- Print out a copy of this protocol to bring with you to Clemson Student Health, Occupational Health, the Emergency Room, or elsewhere, as appropriate.
- Students and staff should contact Will Mayo (office: 864-656-5529) at Clemson Medical Surveillance Program.

If questions or complications arise, you or your physician may consult the Infectious Diseases Society of America’s [Clinical Practice Guidelines for the Management of Cryptococcal Disease](#).

**Treatment:**

The Centers for Disease Control and Prevention (CDC) recommend the following. Individuals who have *C. neoformans* infection need to take prescription antifungal medication for at least 6 months, often longer. The type of treatment usually depends on the severity of the infection and the parts of the body that are affected.

For individuals who have asymptomatic infections (e.g., diagnosed via targeted screening) or mild-to-moderate pulmonary infections, the treatment is usually fluconazole. For individuals who have severe lung infections or infections in the central nervous system (brain
and spinal cord), the recommended initial treatment is amphotericin B in combination with flucytosine. After that, patients usually need to take fluconazole for an extended time to clear the infection. The type, dose, and duration of antifungal treatment may differ for certain groups of people, such as pregnant women, children, and people in resource-limited settings. Some people may also need surgery to remove fungal growths (cryptococcomas).