A Quick Guide to the Significance and Laboratory Identification of *Cryptococcus gattii*

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"News you can use":

- *C. gattii* is an emerging agent of cryptococcosis that is indistinguishable from *C. neoformans* by most routine laboratory tests, e.g. Cryptococcal antigen test, commercial biochemical identification systems for yeast, microscopic morphology, etc.

- *C. gattii* infects both immuno-competent and immuno-compromised individuals, and may be associated with worse clinical outcomes. Due to increasing awareness of *C. gattii* infections, laboratories may be asked to distinguish *C. gattii* from *C. neoformans*

- *C. gattii* can only be differentiated from *C. neoformans* by specific biochemical or molecular testing

Commentary:

Prior to the outbreak of a novel genotype of *Cryptococcus gattii* (VGIIa) in Vancouver Island, B.C. in 1999 (3), *C. gattii* was largely confined to tropical and sub-tropical regions. Since then, *C. gattii* has emerged as an important cause of cryptococcosis in the Pacific Northwest region and a number of novel genotypes have been described (6). The possibility of spread of the VGIIa genotype beyond this region has also recently been noted (7).

The clinical spectrum of cryptococcosis due to *C. gattii* is similar to that of *C. neoformans*, with patients most commonly presenting with respiratory symptoms such as cough and dyspnea (1). However, the ability of this organism to infect immuno-competent individuals has garnered attention from both the medical and scientific communities, as well as from the news media, and *C. gattii* is now a reportable organism in some public heath jurisdictions. Differences in clinical course and treatment outcomes have been suggested for *C. gattii* infections, making it potentially important to distinguish *C. gattii* from *C. neoformans* (2, 8, 9). Interesting epidemiologic differences between these species have been described and are shown in Table 1.

Key laboratory differences and similarities between *C. gattii* and *C. neoformans* are shown in Table 2. Biochemical differentiation of *C. gattii* isolates is based on the ability of *C. gattii,* but not *C. neoformans*, to utilize glycine as a sole carbon and nitrogen source in the presence of canavanine. Thus, *C. gattii* will grow on Canavanine Glycine Bromothymol blue (CGB) agar, turning the medium from green to blue; isolates of *C. neoformans* will not. This medium is commercially available, but is also inexpensive and relatively simple to prepare (5). However, CGB agar needs to be incubated for up to 5 days at 30°C, impacting the turn-around-time of results. Only presumptive isolates of *C. neoformans/gattii (*i.e. *Cryptococcus* isolates confirmed to be positive for melanin production using Caffeic acid disks or Birdseed agar) should be tested on CGB agar as other yeasts can also be CGB-positive, but will be negative for melanin production e.g. *C. laurentii*, *Trichosporon mucoides* (4).

For laboratories with DNA sequencing capabilities, commonly used molecular targets for the detection of fungi from sterile sites by universal PCR (e.g. 28S rDNA) may fail to differentiate between these two species, depending on the region that is sequenced. However, a number of molecular targets have been described that successfully differentiate *C. gattii* from *C. neoformans*. These include the D2 region of 28S rDNA, the rDNA IGS region, and *CAP59* (4). Although PCR-based methods have a rapid turn-around-time, they are expensive and beyond the capabilities of most laboratories, but are available as commercial reference laboratory tests. Because serological testing of isolates to differentiate *C. gattii* (serotypes B and C) from *C. neoformans* (serotypes A and D) is no longer commercially available, laboratories must choose between a biochemical or molecular approach, or a combination thereof, to differentiate these species.

Increasing awareness of *C. gattii* amongst clinicians and the public health community poses a challenge for laboratories. Laboratories should recognize that *C. gattii* cannot be differentiated from *C. neoformans* by conventional laboratory methods, and should therefore consider implementation of specific testing for *C. gattii (as described above)*. If such an approach is not feasible, laboratories should instead consider pursuing definitive identification at an appropriate reference laboratory.

Table 1. Epidemiological and clinical differences between *C. gattii* and *C. neoformans*

Characteristic	***Cryptococcus gattii***	***Cryptococcus neoformans***
Ecological Niche	*Eucalyptus* trees; Native trees of the Pacific NW (e.g. Douglas fir)	Bird Guano
Location	Tropical & subtropical; Pacific NW; possibility for further spread	Worldwide
Immune Status of Host	Immuno-competent (>50%)	Immuno-compromised (>80%)
Lung Manifestations	Commonly nodules	Commonly infiltrates
Brain Lesions	More common	Less common
Hospital Stay and Duration of Therapy	Longer	Shorter

Table 2. Laboratory tests for the differentiation of *C. gattii* from *C. neoformans*

Test	Differentiates	Does not differentiate
Serum tests: a) *Cryptococcus* antigen b) β-1-3-D-glucan		 X (positive for both species) X (negative for both species)
Direct specimen stains: a) Gram b) India Ink c) Calcofluor d) Mucicarmine e) GMS		 X X X X X
Appearance: a) Colony morphology b) Cornmeal-Tween 80 morphology c) Temperature and media requirements		 X X X
Commercial biochemical identification systems e.g. VITEK 2 ID-YST, MicroScan		X
Biochemical tests: a) Urease b) Melanin production (phenyloxidase) c) CBG agar	 X	 X (positive for both species) X (positive for both species)
Serological typing of isolates	X (no longer commercially available)	
PCR	X (target-dependent)	

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