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Cross-Reacting *Ustilago maydis* Causing False-Positive Cryptococcal Antigen Test Results

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Cryptococcus neoformans is a yeast within the division Basidiomycota that may cause pulmonary and central nervous system (CNS) disease. Latex agglutination (LA) and lateral flow assay (LFA) for detection of cryptococcal polysaccharide antigen are sensitive and specific tests for diagnosis of invasive disease (1, 2); however, cross-reactivity has been described with *Trichosporon asahii* (3). Our objective was to determine if there is cross-reactivity with other Basidiomycete yeasts, including rare agents of human disease such as *Rhodotorula*, *Sporobolomyces*, and *Ustilago* spp. (4–8).

Clinical isolates of *Trichosporon*, *Rhodotorula*, *Sporobolomyces*, and *Ustilago* spp. were retrieved from the Quebec provincial reference laboratory (LSPQ). *Sporobolomyces*, *Trichosporon asahii*, *Rhodotorula glutinis*, *R. minuta*, and *R. mucilaginosa* isolates were taxonomically confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Vitek MS; bioMérieux, Inc., Saint-Laurent, Quebec, Canada). *Ustilago* spp. and *Rhodotorula slooffiae* were confirmed by sequencing their ribosomal DNA D_1-D_2 and internal transcribed spacer (ITS) regions. *Cryptococcus neoformans* (LSPQ-16-A479650) and *Candida albicans* (ATCC 60433) strains were used as positive and negative controls, respectively. Growth in Sabouraud dextrose broth was confirmed by measuring the increase in optical density after 24 h (DensiCHEK Plus; bioMérieux, Inc.). Turbidity was adjusted to a 0.5 McFarland (MF) standard equivalent, and organism count/ml was determined by a hemocytometer. This turbidity corresponded to an average of 3.2 organisms/ml (standard deviation, 0.9 organisms/ml), which is similar to the organism burden reported to occur in the cerebrospinal fluid of patients with cryptococcal meningitis (9). Serial 2-fold dilutions were performed to determine the cutoff for positivity.

LA testing was performed using the CALAS cryptococcal antigen latex agglutination kit (Meridian Bioscience, Inc., Cincinnati, OH, USA). Flocculation was graded on a scale from 0 to 4+, with 2 or higher representing a positive result. LFA was performed using the IMMY cryptococcal lateral flow assay (Immuno-Mycologics, Inc., Norman, OK, USA). Both assays were performed per the manufacturers' instructions, and assessment was made in a blind fashion. In total, 23 samples were tested, including 9 isolates of *Rhodotorula* spp., 5 isolates of *Sporobolomyces salmonicolor*, 3 isolates of *Trichosporon asahii*, and 3 isolates of *Ustilago maydis* (Table 1). Only *C. neoformans*, *Trichosporon*, and *Ustilago* isolates were unequivocally positive by both assays. One *Rhodotorula mucilaginosa* isolate and one *Sporobolomyces salmonicolor* isolate had discordant results; these isolates were weakly positive by the LA assay at titers of 1:1 and 1:4, respectively, but were negative by the LFA. All other isolates had concordant results between assays

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TABLE 1 Interassay agreement

Sample group	No. of isolates	Cryptococcal antigen latex test		Cryptococcal LFA	
		Result	Titer	Result	Titer
Sabouraud dextrose broth (negative control)	NA[a]	1+	NA	−	NA
C. albicans ATCC 60433 (negative control)	1	1+	NA	−	NA
C. neoformans (positive control)	1	4+	1:1,024	+	1:256
Rhodotorula glutinis	1	1+	NA	−	NA
Rhodotorula minuta	2	1+	NA	−	NA
Rhodotorula mucilaginosa[b]	5	1+	NA	−	NA
Rhodotorula slooffiae	1	1+	NA	−	NA
Sporobolomyces salmonicolor[c]	5	1+	NA	−	NA
Trichosporon asahii	3	4+	1:32	+	1:32
Ustilago maydis	3	4+	1:32	+	1:16

[a]NA, not applicable.
[b]A single *Rhodotorula mucilaginosa* isolate was weakly positive by the cryptococcal antigen latex test at a titer of 1:1.
[c]A single *Sporobolomyces salmonicolor* isolate was weakly positive by the cryptococcal antigen latex test at a titer of 1:4.

at a 0.5 MF standard equivalent, although some isolates had different cutoffs for positivity (Table 1).

Our findings demonstrate that unlike *Rhodotorula* and *Sporobolomyces* spp., *Trichosporon asahii* and *Ustilago maydis* yield positive results in cryptococcal antigen assays at clinically relevant concentrations, with excellent interassay agreement. The plant pathogen *Ustilago* (the agent of corn smut) and the closely related genus *Pseudozyma* are ubiquitous in the environment and are commonly regarded as laboratory contaminants. However, these organisms can be transmitted by the airborne route and have occasionally caused fungemia and central venous catheter infections in humans (5, 7, 10–16).

In conclusion, our results validate the cross-reactivity of *Trichosporon asahii* as previously reported and help delineate the limitations in specificity of cryptococcal antigen testing for the detection of other basidiomycetous yeasts. Although this study is limited to *in vitro* observations, our results suggest that these tests should not be used as an adjunct to diagnose invasive infections caused by *Rhodotorula* or *Sporobolomyces* species but may be considered for *Trichosporon asahii* and *Ustilago maydis*. The fact that not all Basidiomycetes tested produced a cross-reaction in the two assays suggests there is no conserved cross-reacting antigen produced by all members of the division Basidiomycota.

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M.P.C., T.T.N., and L.O.P. performed literature searches, devised the experiments, and drafted the manuscript. P.J.D. and D.C.S. were responsible for the overall content.

We declare that we have no conflicts of interest.

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