



# 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<sub>1</sub>-D<sub>2</sub> 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 <sup>a</sup>	1+	NA	–	NA
<i>C. albicans</i> ATCC 60433 (negative control)	1	1+	NA	–	NA
<i>C. neoformans</i> (positive control)	1	4+	1:1,024	+	1:256
<i>Rhodotorula glutinis</i>	1	1+	NA	–	NA
<i>Rhodotorula minuta</i>	2	1+	NA	–	NA
<i>Rhodotorula mucilaginosa</i> <sup>b</sup>	5	1+	NA	–	NA
<i>Rhodotorula slooffiae</i>	1	1+	NA	–	NA
<i>Sporobolomyces salmonicolor</i> <sup>c</sup>	5	1+	NA	–	NA
<i>Trichosporon asahii</i>	3	4+	1:32	+	1:32
<i>Ustilago maydis</i>	3	4+	1:32	+	1:16

<sup>a</sup>NA, not applicable.

<sup>b</sup>A single *Rhodotorula mucilaginosa* isolate was weakly positive by the cryptococcal antigen latex test at a titer of 1:1.

<sup>c</sup>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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## REFERENCES

- Hansen J, Slechta ES, Gates-Hollingsworth MA, Neary B, Barker AP, Bauman S, Kozel TR, Hanson KE. 2013. Large-scale evaluation of the immuno-mycologicals lateral flow and enzyme-linked immunoassays for detection of cryptococcal antigen in serum and cerebrospinal fluid. *Clin Vaccine Immunol* 20:52–55. <https://doi.org/10.1128/CVI.00536-12>.
- Kauffman CA, Bergman AG, Severance PJ, McClatchey KD. 1981. Detection of cryptococcal antigen. Comparison of two latex agglutination tests. *Am J Clin Pathol* 75:106–109. <https://doi.org/10.1093/ajcp/75.1.106>.
- McManus EJ, Jones JM. 1985. Detection of a *Trichosporon beigelii* antigen cross-reactive with *Cryptococcus neoformans* capsular polysaccharide in serum from a patient with disseminated *Trichosporon* infection. *J Clin Microbiol* 21:681–685.
- Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, European Society of Clinical Microbiology and Infectious Diseases Fungal Infection Study Group, European Confederation of Medical Mycology. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. *Clin Microbiol Infect* 20(Suppl 3):S76–S98. <https://doi.org/10.1111/1469-0691.12360>.
- McGhie TA, Huber TW, Kassis CE, Jinadatha C. 2013. *Ustilago* species as a cause of central line-related blood stream infection. *Am J Med Sci* 345:254–255. <https://doi.org/10.1097/MAJ.0b013e31826f56ed>.

6. Moore M, Russell WO, Sachs E. 1946. Chronic Leptomeningitis and ependymitis caused by *Ustilago*, probably *U. zeae* (corn smut). *Am J Pathol* 22:761–777.
7. Patel R, Roberts GD, Kelly DG, Walker RC. 1995. Central venous catheter infection due to *Ustilago* species. *Clin Infect Dis* 21:1043–1044. <https://doi.org/10.1093/clinids/21.4.1043>.
8. Stewart E, Waldman S, Sutton DA, Sanders C, Lindner J, Fan H, Wiederhold NP, Thompson GR, III. 2016. *Ustilago echinata*: infection in a mixed martial artist following an open fracture. *Mycopathologia* 181:311–314. <https://doi.org/10.1007/s11046-015-9967-1>.
9. Jarvis JN, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC, Longley N, Muzoora C, Phulusa J, Taseera K, Kanyembe C, Wilson D, Hosseinipour MC, Brouwer AE, Limmathurotsakul D, White N, van der Horst C, Wood R, Meintjes G, Bradley J, Jaffar S, Harrison T. 2014. Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis* 58:736–745. <https://doi.org/10.1093/cid/cit794>.
10. Herb A, Sabou M, Delhorme JB, Pessaux P, Mutter D, Candolfi E, Letscher-Bru V. 2015. *Pseudozyma aphidis* fungemia after abdominal surgery: first adult case. *Med Mycol Case Rep* 8:37–39. <https://doi.org/10.1016/j.mmcr.2015.03.001>.
11. Joo H, Choi YG, Cho SY, Choi JK, Lee DG, Kim HJ, Jo I, Park YJ, Lee KY. 2016. *Pseudozyma aphidis* fungaemia with invasive fungal pneumonia in a patient with acute myeloid leukaemia: case report and literature review. *Mycoses* 59:56–61. <https://doi.org/10.1111/myc.12435>.
12. Lin SS, Pranikoff T, Smith SF, Brandt ME, Gilbert K, Palavecino EL, Shetty AK. 2008. Central venous catheter infection associated with *Pseudozyma aphidis* in a child with short gut syndrome. *J Med Microbiol* 57:516–518. <https://doi.org/10.1099/jmm.0.47563-0>.
13. Orecchini LA, Olmos E, Taverna CG, Murisengo OA, Szuzs W, Vivot W, Cordoba S, Bosco-Borgeat ME, Montanaro PC. 2015. First case of fungemia due to *Pseudozyma aphidis* in a pediatric patient with osteosarcoma in Latin America. *J Clin Microbiol* 53:3691–3694. <https://doi.org/10.1128/JCM.01095-15>.
14. Pande A, Non LR, Romee R, Santos CA. 6 March 2017. *Pseudozyma* and other non-*Candida* opportunistic yeast bloodstream infections in a large stem cell transplant center. *Transpl Infect Dis* <https://doi.org/10.1111/tid.12664>.
15. Prakash A, Wankhede S, Singh PK, Agarwal K, Kathuria S, Sengupta S, Barman P, Meis JF, Chowdhary A. 2014. First neonatal case of fungaemia due to *Pseudozyma aphidis* and a global literature review. *Mycoses* 57:64–68. <https://doi.org/10.1111/myc.12098>.
16. Siddiqui W, Ahmed Y, Albrecht H, Weissman S. 2014. *Pseudozyma* spp catheter-associated blood stream infection, an emerging pathogen and brief literature review. *BMJ Case Rep* 2014;pii=bcr2014206369. <https://doi.org/10.1136/bcr-2014-206369>.