

Figure 1. Diagram of the Dynamiker GM (LFA)

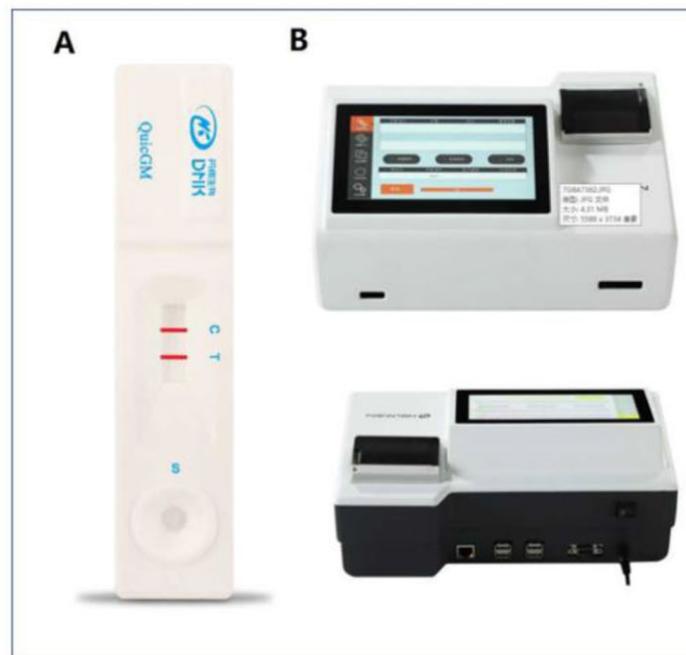


Figure 2. Dynamiker GM (LFA) (A) and its reader (B)

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Cryptococcosis screening and isolates characterization in asymptomatic people living with HIV in Kinshasa, DRC

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Objectives: Cryptococcal meningitis (CM) is a life-threatening invasive mycosis affecting people living with HIV (PLHIV) and has a high prevalence and case fatality rate in sub-Saharan Africa. While most PLHIV presenting CM are symptomatic, the asymptomatic ones are diagnosed following routine screening tests indicated for all advanced PLHIV (CD4 <200/ μ L). We, therefore, hypothesized that asymptomatic CM patients would be infected with different *Cryptococcus* spp. strains than those in symptomatic CM patients (referring to the parallel study conducted in the same clinics). This study describes the prevalence of serum and meningeal cryptococcosis in asymptomatic PLHIV presenting a CD4 count of <200 cells/ μ L in the screening context. We then characterized and determined the antifungal susceptibility of *Cryptococcus* spp. strains isolated from these patients.

Methods: We performed cross-sectional screening for serum cryptococcal antigen (CrAg) in ambulant PLHIV with <200 CD4/ μ L in three clinics in Kinshasa (DRC). Lumbar puncture was indicated in positive patients to exclude a meningeal location for therapeutic purposes. The resulting cerebrospinal fluid (CSF) was then analyzed for CrAg and *Cryptococcus* spp. was isolated and characterized by MALDI TOF MS, serotyping PCR, ITS sequencing, and multilocus sequence typing (MLST). In addition, the EUCAST E.Def.7.3.2 microdilution procedure was followed to determine the susceptibility of strains to antifungal agents.

Results: A total of 47 PLHIV out of 262 included were tested positive for serum CrAg (19%, 95% CI: 14.2–24.3) from which 46.8% (22/47) had a high antigenic titer ($\geq 1/160$). The prevalence of asymptomatic CM was then estimated at 50% in CrAg serum-positive patients who consented to lumbar puncture (19/38). Although the female proportion included in the study was higher than that of men, serum CrAg was more positive in men (21.4%, 18/84 men included) than in women (18.0%, 29/161

women included). While the mean CD4 count of CrAg serum-positive patients were significantly lower than that of negative patients ($P < .05$), the median viral load between the two patient groups was approximately similar ($P > .05$). Only four CSF samples were positive in culture for *Cryptococcus* spp. and were all characterized as *Cryptococcus neoformans*/serotype A.

At this stage, two isolates have been analyzed using the ISHAM MLST scheme and two different sequence type (ST) profiles were identified, namely: ST93 and ST63. While ST93 is the main *Cryptococcus neoformans* profile described in Congolese (DRC) PLHIV with CM, ST63 has not yet been identified in the DRC before. Of note, epidemiological and clinical specificities of ST63 have so far been poorly characterized in the literature. Susceptibility testing against the major antifungals and the MLST typing of the two remaining strains are still ongoing.

Conclusions: The prevalence of cryptococcosis should not be neglected among asymptomatic PLHIV in the DRC, to meaning that screening and preventive treatment measures should be integrated into the national policy for HIV management and related diseases. For the rest of the analyses still in progress, conclusions can only be drawn once they have been fully finalized.

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Spread of *sporothrix brasiliensis* from the sneeze of infected cats: a potential novel route of transmission

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Objectives: Cat-transmitted sporotrichosis (CTS), caused by *Sporothrix brasiliensis*, is an emerging fungal disease that has become a major public health concern in Brazil. Transmission of CTS usually occurs through the implantation (e.g., scratches or bites) of infectious yeast from feline *Sporothrix* lesions. Recent reports on transmission events have suggested that *S. brasiliensis* might be transmitted through feline respiratory droplets created while sneezing. The aim of our study is to determine whether infectious respiratory secretions are expelled when cats with sporotrichosis sneeze.

Methods: We collected respiratory secretions expelled while sneezing from 28 cats diagnosed with sporotrichosis. We placed a Mycosel agar plate, a fungal culture medium, in front of the animals' nostrils and used a nasal swab to stimulate sneezing (Fig. 1). Samples were incubated at 28-30°C for 4 weeks in the Mycology laboratory of Hospital de Clinicas. Molecular identification of the isolates was performed by sequencing the calmodulin gene. The infected cats enrolled in the study were subsequently treated at the School Veterinary Clinic of the Pontifical Catholic University of Paraná, a referral hospital for the treatment of feline sporotrichosis.

Results: Out of the 28 respiratory samples collected, 20 (70%) had evidence of fungal growth morphologically consistent with *Sporothrix*. Sequencing of all isolates identified *Sporothrix brasiliensis* (Fig. 2).

Conclusion: We identified a possible novel route of transmission of *Sporothrix* spp. through infectious feline respiratory secretions expelled during sneezing. The respiratory droplets created by a sneeze could contain viable *Sporothrix* yeast that could infect humans and other animals after mucosal exposure. One health partner and collaborator such as veterinarians, physicians, health authorities, epidemiologists, and fungal disease researchers should be made aware of the potential spread of *Sporothrix* through respiratory droplets and sneezing to prevent and control the further spread of CTS. To prevent cat-to-human transmission of *Sporothrix brasiliensis*, personal protective equipment (PPE) should be worn while handling a cat with suspected sporotrichosis. Veterinarians, veterinary clinic employees, students, and pet shop owners are at increased risk due to their professions. Veterinary care frequently involves procedures that encourage respiratory droplets (e.g., nasal swabs); restraint and other close contact may directly expose staff to infectious secretions. Because this study identified viable yeast in respiratory droplets from sneezing, decontamination and disinfection of exposed surfaces is increasingly important, as surfaces and objects can serve as fomites for *Sporothrix*. Physicians who diagnose and treat human cases of sporotrichosis should be aware of this new transmission method to improve clinical suspicion, diagnosis, and treatment for sporotrichosis. Approximately half of the human patients with conjunctival sporotrichosis did not report experiencing traumatic injury from cats; mucosal exposure to infectious yeast is a likely alternative transmission method.

Figure 1. Collection and isolation of cat's sneeze with sporotrichosis. Collecting technique of cat's sneeze (A). Isolation of the mycelial phase of *Sporothrix brasiliensis* after cat sneezing directly into the Mycosel plate (B)

