

Title: DEVELOPMENT OF DNA MICROARRAY PLATFORMS FOR DIAGNOSIS OF
CRYPTOCOCCAL MENINGITIS

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Abstract:

The proper and fast diagnosis of cryptococcal meningitis and the *Cryptococcus* species are the main steps to initiate the antifungal therapy for these patients. The aim of this study was to develop DNA microarray platforms for the identification of the two *Cryptococcus* species, *gattii* and *neoformans*, directly from clinical samples of cerebrospinal fluid (CSF) of patients with meningitis. CSF samples were obtained from patients admitted in the Clinical Hospital of the University of Campinas with diagnosis of cryptococcal meningitis (n=6) by the microbiology laboratory. CSF samples that resulted negative in cultures for bacteria and fungi (n=31) and bacteria-positive CSF samples (n=13) were used as control of the reactions. Three protocols of CSF lysis for DNA extraction were evaluated using CTAB (Cetyltrimethylammonium bromide) solution, alkaline wash solution and 1X PBS, however the protocol tested with alkaline washing solution was more sensitive. Fungal DNA was submitted to amplification using primers to amplify the ITS region of the ribosomal DNA and the CAP59 region of the polysaccharide capsule. PCR products were subjected to identification of species by the two DNA microarray platforms. The two platforms were previously tested using DNA from isolates, ATCC 90113 *C. neoformans* and LIF 708 *C. gattii* that grew directly from the culture. DNA sequencing was also performed for species determination. Five *C. neoformans* and one *C. gattii* isolated from CSF were tested and precisely identified by the CAP59 platform, but not from the ITS platform that only identified the genus *Cryptococcus* but not the species. The microarray results for *Cryptococcus* had 100% agreement with the identification by DNA sequencing. New CSF samples need to be collected and tested on the platforms in order to evaluate the efficiency and sensitivity in differentiate *C. gattii* and *C. neoformans* species in clinical specimens.

Keywords: *Cryptococcus neoformans*, *Cryptococcus gattii*, molecular diagnosis, cerebral spinal fluid, microarray

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