

O0850 **Discrimination between *Cryptococcus neoformans* var. *neoformans* and *C. neoformans* var. *grubii* using MALDI-TOF MS**

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**Background:** Differentiation between both varieties of *Cryptococcus neoformans* (*C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii*) is important to define the epidemiology of the infection. So far, discrimination between both species relies on either phenotypic methods or on DNA sequencing analysis. Both techniques are cumbersome and time consuming. In this study we attempted the discrimination between *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* using MALDI-TOF MS and an in-house library built with well-characterized isolates from clinical samples.

**Materials/methods:** All the *Cryptococcus* isolates included in the study were identified by DNA sequencing analysis of the ITS1-5.8S-ITS2 region. An in-house database was constructed with 11 *C. neoformans* var. *neoformans* and 12 isolates *C. neoformans* var. *grubii* isolates from clinical samples adding the spectra following manufacturer's instructions.

Forty-seven *Cryptococcus* isolates were then blindly analyzed using an LT Microflex benchtop MALDI-TOF mass spectrometer (Bruker Daltonics) and the in-house library. Biomass was picked from the colonies, re-suspended in 300 µl water HPLC-grade and 900µl ethanol and submitted to 5 min vortexing with 0.5 mm glass beads. After a brief centrifugation, the supernatant was discarded and the pellet allowed drying completely. A standard protein extraction step with formic acid and acetonitrile was performed and 1µl of the supernatant was placed onto the MALDI target plate in triplicates for spectrum achievement using MALDI-TOF MS.

**Results:** Overall, 95.7% of the isolates were correctly identified by MALDI-TOF MS. Only two *C. neoformans* var. *neoformans* isolates were misidentified as *C. neoformans* var. *grubii*. Besides, 44/47 isolates were identified with score values  $\geq 1.8$  which indicates a high confidence level. Only one isolate obtained a score value below 1.6.

**Conclusions:** Our results indicate that MALDI-TOF MS could be a rapid and reliable tool for the correct discrimination between *C. neoformans* varieties. The implementation of a well-fed in-house library where all *C. neoformans* are well represented will surely allow an accurate discrimination among varieties in the near future.