

**TITLE:** PERFORMANCE OF MICROFLEX LT AND VITEK MS PLATFORMS IN MASS SPECTROMETRY IDENTIFICATION OF *CRYPTOCOCCUS NEOFORMANS* AND *CRYPTOCOCCUS GATTII* GENOTYPES.

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**ABSTRACT:**

*Cryptococcus neoformans* and *Cryptococcus gattii* are globally responsible for almost one million cryptococcosis cases. Phylogenetic studies using molecular typing allowed the division of the two species into eight molecular types: each species has four genotypes, *C. neoformans* (VNI to VNIV) and *C. gattii* (VGI to VGIV). MALDI-TOF mass spectrometry (MS) has emerged as a useful technique for rapid and accurate pathogen identification in clinical laboratories, moreover, most of the studies that have evaluated MALDI-TOF MS for identification of clinically relevant species of *Cryptococcus* have used only the Bruker platform instrument and databases (eg Biotyper). This study aims analyze critically the performance of MALDI-TOF (bioMérieux, Marcy-L'Etoile, France) by Vitek MS platform for the separation of *C. neoformans* and *C. gattii* genotypes. Spectral profile database, in house, was constructed identifying the genotypes. We used 35 clinical samples of *Cryptococcus spp.* and 8 standard strains of the respective genotypes (VNI-VNIV / VGI-VGIV). The standard phenotypic methods used for the identification of clinical samples were consistent with the genus *Cryptococcus* [urease and Niger agar and chemotyping (CGB)]. The molecular methodology used DNA extractions and subsequent double restriction analysis, with the enzymes Sau96I and HhaI, in the *URA5* gene sequences. The procedure allowed generating maps with different restriction profiles to compare with the profiles generated by the standard strains. The results of the clinical samples evaluated revealed correct identification of the genotype in comparison with the standard strains. Eight reference strains were used as standard for the construction of our database library with reference spectra. The spectra resulting of these samples were compared to the Saramis Premium software database in its default configuration. The original Vitek MS database allowed only the identification of genus and species in most cases, while with the ERs (Super Spectros) created, besides the identification of genus and species, the identification of genotypes was allowed (n=35). We conclude that the newly created in-house ERs will be useful to complete the identification of genotypes of *Cryptococcus spp.* These results obtained by the mass spectrometry technique are relevant for epidemiological and therapeutic purposes obtaining a fast and reliable identification of the species of these emerging pathogens.

**KEYWORDS :** *Cryptococcus* spp., Genotypes, MALDI-TOF, Vitek MS

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