

TITLE: A IMPROVED PROCEDURE FOR RAPID IDENTIFICATION OF YEASTS USING MALDI-TOF MS.

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ABSTRACT:

Introduction: The prompt and accurate identification of fungi is of major importance to support the correct use of antifungal. The recent development of yeast identification by mass spectrometry significantly decreases the time for definitive identification and represents an advance in clinical microbiology. Protein extraction for smear preparation and identification by the MALDI-ToF MS instrument can be performed on the target plate or in a microtube. Tube extraction requires longer hands on time while on plate extraction is faster and simpler. Consequently, extraction on plate is the preferred method for the clinical microbiology routine, but sometimes fails to generate adequate results. The objective of the present study was to compare two on plate extraction methods for improved yeast identification. **Methods:** A total of 68 yeast strains were subcultured on Chromogenic Candida agar, incubated for 24 hours in ambient air and were tested using the Microflex system (Brucker Daltonics). Two extraction methods were performed for each strain. Duplicate smears for each extraction method were prepared from the same single colony. In the regular method (method 1), smears were overlaid with 1 µl of 70% formic acid, they were allowed to dry and subsequently 1 µl of matrix solution was added. They were let to dry again and were subsequently analyzed using the Microflex system. In the new procedure, after drying the formic acid solution, another drop (1 µL) of 70% formic acid was added to each smear. Smears were allowed to dry and subsequently 1 µl of matrix solution was added. They were let to dry again and were subsequently analyzed using the Microflex system. **Results:** A total of 68 yeast strains were analyzed: being 59 *Candida* spp. (28 *Candida parapsilosis*, 12 *Candida albicans*, 8 *Candida tropicalis*, 5 *Candida glabrata*, 3 *Candida guilliermondii*, 2 *Candida kefyr*, 1 *Candida haemulonii*), 7 *Trichosporon* strains and 2 *Cryptococcus* strains. The scores observed after extraction methods 1 and 2 were, respectively, score ≥ 2.0 (high degree of confidence): 10 (14.7%) and 41 (60.3%); score between 1.7 and 1.99 (low confidence level): 18 (26.5%) and 27 (39.7%). By extraction method 1, it was impossible to identify 58.8% of the strains (score < 1.70), however all the strains obtained score ≥ 1.70 by extraction method 2. The proposed extraction method provides a simple, fast and effective procedure to improve the identification of yeasts in the routine of a clinical microbiology laboratory.

Keywords: yeasts identification, mass spectrometry, MALDI-ToF Brucker, protein extraction, chromogenic agar

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