TITLE: EVALUATION OF FILTER PAPER TO TRANSPORT BACTERIA FOR MALDI-TOF/MS ANALYSIS

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ABSTRACT: Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been successfully used to identify bacteria in clinical microbiology laboratories. Small laboratories do not have an easy access to the benefits of MALDI-TOF due to equipment costs and have to send the isolates to be identified in central labs. Transportation of bacteria is related to biological risks and it is important to develop alternatives to minimize these risks. This study aimed to evaluate the transport of bacteria in filter paper for MALDI-TOF analysis. A total of 74 isolates (50 gram negative and 24 gram positive) of 19 species were evaluated. Bacterial inactivation was assessed by 70% ethanol for different times (5, 10 and 15 min.). After inactivation, the mixture was centrifuged at 13.000 rpm for 3 minutes, the supernatant was removed and the residual ethanol was evaporated at room temperature. The pellet was impregnated in filter paper disks for transportation. The pellet was also inoculated in solid and liquid media. One paper disk was left at room temperature for around 60 minutes and the other disk was kept at room temperature for 8 days. The disks were submitted to protein extraction with 150 µl of 70% formic acid and 150 µl of acetonitrile in an eppendorf tube by vortexing for 20 seconds. After 3 min of centrifugation at 13.000 rpm, 1 µl of the supernatant was spotted onto the target plate and overlaid with 1 µl of alpha-cyano-4-hydroxycinnamic acid (α-CHCA). MALDI-TOF MS analysis was performed in a Bruker AutoFlex LT mass spectrometer (Bruker Daltonics, Billerica, MA) using the Bruker MALDI BioTyper System (v3.1 Bruker Daltonics, Inc.). The Bruker MALDI Biotyper interpretative criteria were used as follows: ≥ 2.3 (+++), ≥ 2 to 2.29 (++), ≥ 1.7 to 1.99 (+) and < 1.7 (-). Seventy-two (97.3%) isolates transported in filter paper were correctly identified as follows: 68.9% (n = 51) with scores > 2 (reliable identification to species level) and 28.45% (n = 21) with scores between 1.7 and 1.99 (reliable identification to genus level). Sensitivity was 97.3% and specificity of 100%. The isolates in filter paper that were kept at room temperature for 8 days presented no score reduction. The time of 15 min in 70%¨ethanol was enough to inactivate all isolates. Our results indicate that inactivated bacteria in paper filter can be transported for MALDI-TOF identification.

Keywords: MALDI-TOF, filter paper, biohazard

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