Direct Transfer (DT) and extended Direct Transfer (eDT) Methods: Bacteria and Yeast

Direct Transfer: Approximately 95% of routinely isolated bacteria can be analyzed by MALDI using the DT method and achieve a genus species level score. The success of this technique depends largely due to the thinness of the smear (technologist) and the "thickness" of the cell wall. The higher the percentage of peptidoglycan in the cell wall, the more difficult it can be for the nitrogen laser (used by the Biotyper) to penetrate. Some gram positive bacteria cell walls are 90% peptidoglycan! Gram negative bacteria have very thin cell walls (5-20% peptidoglycan) and will easily be analyzed easily by the DT method. So what can we do to help break the cell walls when we are analyzing those tough gram positive bacteria and yeast?

Extended Direct Transfer: Some gram positive bacteria will not achieve a genus species level score when the DT method is used. For example, Micrococcus, Rhodococcus, Corynebacteria, and all yeast have very thick peptidoglycan walls and will rarely achieve a genus species level score if tested using the DT method. The eDT method can be used on these gram positive bacteria or yeast, which is simply adding a 1 µL of 70% Formic Acid overlay to the DT. The Formic Acid will "digest" the cell wall, allowing the matrix to surround and help ionize the ribosomal proteins used for identification. Commonly, laboratories will smear (2) spots (only touching the colony one time creating a dilution in the second spot) and perform the eDT on the first spot and leave the second spot free of formic acid (DT).

Materials Needed:

1. MALDI Biotyper Target
2. Bacterial Test Standard (BTS)
3. HCCA (matrix)
4. 70% Freshly prepared Formic Acid
5. Toothpicks or another transfer device
6. Eppendorf Brand 1 µL Pipette/Tips
7. MALDI Biotyper Target Worksheet
8. Fresh Microorganism: overnight growth should be used for routine microorganism identification; slow-growing bacteria may need to incubate for several days before testing. Do not use organisms that have been stored at 4°C or lower, as this has a negative impact on quality of spectra and reproducibility; storing plates at room temperature for several days is acceptable. Different media types and growth temperatures have little effect on results

MALDI Target Sample Preparation:

1. Prepare MALDI Target Worksheet with appropriate sample identification, allowing two target spots for BTS (QC) and each isolate to be analyzed (run isolate in duplicate)
2. Pipet 1 µL of BTS the first (2) spots of the target to be used; **Apply HCCA immediately after BTS is dry; prolonged expose to air will result in protein oxidization**

3. Touch single colony with toothpick **and smear a thin layer of microorganism** onto first spot. **GO DIRECTLY TO SECOND SPOT** and smear the same microorganism, using the same toothpick (trying to achieve a very thin layer of microorganism).
4. Apply 1µL of 70% Formic Acid to the FIRST Spot (heavier); dry completely
5. Apply 1µL of HCCA to each spot on target
6. Allow to dry at room temperature; perform MALDI Biotyper measurements

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