Formic Acid/Ethanol Tube Extraction (TE) Method

Principle: Some microorganisms, such as yeast, naturally have very thick cell walls. Older isolates, such as Staphylococcus *sp.* and Corynebacteria *sp*., may also acquire thicker cell walls during the aging process. It may be necessary to break down the cell wall and separate the ribosomal proteins prior to spectra analysis. If bacteria and yeast do not yield a desired score using the (eDT) procedure, the (TE) procedure may be used as a last resort.

Materials Needed :

1. Eppendorf 1.5 µL microfuge tubes
2. Eppendorf pipettes/tips
3. Microfuge
4. Toothpicks (or applicable transfer device)
5. Vortex
6. Microfuge tube rack
7. HCCA
8. BTS
9. Target
10. Ultra-Pure Water, HPLC/MS Grade
11. Ethanol, 100% HPLC/MS Grade
12. Formic Acid, 70% HPLC/MS Grade
13. Acetonitrile, 100 % HPLC/MS Grade
14. Fresh Microorganism: overnight growth should be used for routine microorganism identification; slow-growing bacteria may need to cultivate 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

Tube Extraction Procedure:

1. Add **300 µL** of water to each Eppendorf microfuge tube
2. Transfer a large, single colony of microorganism to the tube (more than one colony may need to be transferred if microorganism is small; chose isolated colonies); Vortex thoroughly
3. Add **900 µL** of Ethanol; Vortex thoroughly
4. Centrifuge at maximum (13,000 to 15,000 rpm) speed for (2) minutes
5. Decant Ethanol; Centrifuge for (2) minutes
6. Remove ALL excess Ethanol with pipette (completely remove all Ethanol; tubes may left at room temperature to complete the evaporation process if necessary)
7. Add **50 µL of 70% Formic Acid** (if only a small amount of microorganism is available decrease the Formic Acid volume to 10 µL)
8. Vortex thoroughly and let stand for approximately 5 minutes

9. Add **50 µL of 100% Acetonitrile** (if only a small amount of microorganism is available decrease the
10. Acetonitrile volume to 10 µL); vortex thoroughly; ***NOTE: the volumes of 70% Formic Acid and Acetonitrile must be equal volumes***
11. Centrifuge at maximum speed (13,000 to 15,000 rpm) for (2) minutes
12. Pipette **1 µL** of supernatant onto steel target; avoid touching pellet at bottom of tube with pipette tip; air dry
13. Overlay with **1 µL** of matrix; air dry (ensure target is completely dry before it is inserted into MBT!)
14. Analyze

*****All statements and applications pertaining to the MBT Biotyper are for research use only***