AFA-based MALDI Biotyper Sample Preparation for Mycobacteria Colonies

INTRODUCTION

MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) continues to be adopted worldwide as a highly effective instrumentation method for microbial identification. MALDI-TOF MS measures the unique molecular fingerprint of an organism. This Application Note updates the use of Adaptive Focused Acoustics® (AFA™) specifically for use with the MALDI Biotyper® for identification of mycobacteria.

Thousands of bacteria, yeasts and fungi have been fully characterized using the MALDI Biotyper platform using on-plate extraction. Mycobacteria and fungi require additional sample preparation in order to extract peptides and proteins to achieve reliable and routine identification. For mycobacteria, it is required to use either the Bruker Myco-Ex protocol (1) or the Covaris truXTRAC™ MALDI-TOF MS Protocol (2) with the Covaris M220 Focused-ultrasonicator (3).

The MALDI Biotyper measures highly abundant proteins that are found in microorganisms. The use of the Covaris M220 and truXTRAC MALDI-TOF MS Protocol 010324 for Mycobacteria Colony Samples enables the microbiology lab to immediately utilize the MALDI Biotyper to test unknown clinical isolates and initiate validation studies.

In many parts of the world, increased migration and immigration are expected to result in an increased incidence of Mycobacterium tuberculosis and also non-TB mycobacteria (NTB). Therefore, the need to utilize existing MALDI Biotyper equipment for more rapid identification of positive cultures is more urgent.

APPLICATION DEFINITION AND CONSIDERATIONS

Conventional identification of mycobacteria from colonies is based on HPLC (mycolic acid), PCR or phenotypic and biochemical identification. Laboratories that already utilize the MALDI Biotyper for general bacterial colony identification can now benefit from improvements in sample preparation enabled by the Covaris truXTRAC MALDI-TOF MS Protocol to accelerate streamlining the sample prep of positive mycobacteria cultures.

Sample preparation for this application includes the following considerations:

- The Covaris M220 instrument may already be in use at your (core molecular biology) facility for other applications, such as DNA shearing for Next-Generation Sequencing (6).
- The MALDI Biotyper database should be the most recent, version 3.0 or higher.
- Cultures need to be freshly positive, grown in advance for batch testing. Do not use "old" positive cultures for evaluation of the Covaris protocol 010324.
- Suspect mycobacteria cultures need to be heat inactivated in water prior to solvent extraction.
- Covaris incorporates heat inactivation into their optimization of solvent extraction.

INSTRUMENT SETUP, LOCATION AND OPERATION

- The Covaris M220 instrument dimensions are 12" W x 17" D x 10" H (30cm x 43cm x 25cm.)
- Power requirements are 100-240 VAC 500 VA, 50-60Hz.
- The M220 instrument includes a notebook computer and SonoLab 7 software.A specific software file (method) is set up in advance for this protocol that assures consistency of applied power, duty factor, cycles per burst, temperature and run time. (Note: other applications for the M220 will have separate files saved that are optimized for specific protocols.)
- All samples must be heat inactivated prior to exposure to the M220 instrument, therefore the M220 does not require operation in the BSL3 facility.

HEAT INACTIVATION

The Bruker Mycobacteria database was developed using extracted peptides and proteins from colony samples that were heated in water prior to extraction. This method results in a specific unique molecular fingerprint for each species. The Covaris protocol is intended to insure the equivalent match of an unknown sample to the database for accurate identification.

- The Bruker MycoEx protocol advises the use of a 300 µl

Eppendorf tube. The Covaris protocol uses a specific 100 µl Eppendorf tube, recommended for optical clarity and low volume of Extraction Solvent used to transfer the sample.

- The Bruker MycoEx protocol and the Covaris protocol advise to perform heat inactivation by boiling for 30 minutes. Individual labs should have current equipment adequate for heating samples. Covaris recommends to use the same apparatus used for heating the samples prior to the MycoEx protocol.

ACOUSTICAL CUVETTE SAMPLE VESSEL FEATURES UPDATE

Covaris developed a dedicated disposable acoustical cuvette for this application, the microTUBE-130 Glass Beads Pre-Slit Screw-Cap 25/pk. (4)

- 25 mg glass beads are pre-installed in each microTUBE.
- The microTUBE liquid capacity is 130 µl.
- Extraction Solvent volume used for this application is 50 µl. The low volume increases peptide concentrations to improve MALDI Biotyper log scores.
- The pre-slit screw cap design allows direct pipet access for adding Extraction Solvent without removing the cap.
- The hub of the Acoustical Cuvette is bar-coded.
- Prep station (5) is designed to hold up to eight microTUBEs.

EXTRACTION CHEMISTRY

- The Covaris protocol 010324 recommends to make up in advance the Extraction Solvent, a mixture of (50% acetonitrile/35% formic acid/15% H_2O). Acetonitrile and formic acid should be HPLC/MS grade.
- EtOH at any concentration is not required.
- The extraction of peptides/proteins from the Mycobacteria sample is achieved by AFA processing 60 seconds per sample with the Extraction Solvent.

M220 INSTRUMENT AND HARDWARE ACCESSORIES REQUIRED

- Covaris M220 Focused-ultrasonicator (p/n 500295) with computer and Software for single sample processing.
- Covaris ME220 Focused-ultrasonicator (p/n 500506) for up to eight sample processing. *
- Covaris part# 520194 startup package includes:
  - M220 Holder XTU (p/n 500414)
  - Holder insert for microTUBE 130 tubes (p/n 500489)
  - Prep Station for 8 microTUBE 130 tubes (p/n 500468)
  - Centrifuge and heat block adaptors for microTUBE 130 tubes (p/n 500406)
  - Acoustical Cuvette microTUBE-130 Glass Beads Pre-Slit Screw-Cap 25/pk (p/n 520199)
  - Eppendorf tubes 0.2 ml PCR grade Eppendorf (p/n 951010006)(25)

M220 FOCUSED-ULTRASONICATOR OPERATION NOTES

SEE COVARIS M-SERIES USER MANUAL (P/N 010157)

- Ensure that the method, a saved file in the Sonolab software is up to date regarding power, duty factor, cycles per burst, temperature and duration.
- The settings should be set to the following: Power 40 W PIP, Duty Factor 50%, Cycles per burst 50, Run time 60 seconds, temperature 18° C.
- Avoid using a saved method file for other applications or previous Mycobacteria protocols.
- 60 second AFA processing per sample.
- AFA is the fastest section of the overall protocol.

SAMPLE HANDLING NOTES

- Colonies should be adequately sampled for 4-8 mg of biomass.
- Avoid sampling the agar media.
- Add 100 µl water to Eppendorf tube.
- Add biomass to Eppendorf tube.
- Perform heat inactivation of the samples by boiling for 30 minutes.
- Cool, centrifuge, decant water carefully to not disrupt the pellet.
- Add 50 µl Extraction Solvent to transfer the pellet to the Acoustical Cuvette

MALDI BIOTYPER NOTES

- Set up target plate within one hour after AFA processing.
- Test four replicate spots per sample.
- Follow Bruker instructions for application of matrix

DATA

Table 2. Using Covaris truXTRAC™ MALDI-TOF MS Protocol (p/n 010324.) Bruker MALDI Biotyper Scores from *Mycobacterium smegmatis culture after a two-day growth on Middlebrook agar plates.*

#	Covaris truXTRAC	Bruker MycoEx
1	2.367	2.252
2	2.417	2.210
3	2.397	2.046
4	2.248	2.259
5	2.327	2.302
6	2.432	2.274
7	2.480	2.210
8	2.314	2.278
Average	**2.373**	**2.229**
CV%	3.13%	3.61%

DISCUSSION

For your high incidence mycobacteria isolated from positive cultures, log scores should be >2.0 in most cases. Consult with your supervisor regarding how to report MALDI Biotyper results in the >1.9 range and in the >1.7 range, based on your institution practices and recommendations from Bruker.

SUMMARY

The truXTRAC MALDI-TOF MS Heat-inactivated Preparation Protocol for Mycobacteria Colony Samples and Analysis on the Bruker MALDI Biotyper should work immediately on the first day of operation. This enables preparing samples from actual clinical positive cultures, in addition to known, in-house previously identified strains. Validation requirements will vary by institution.

OTHER APPLICATIONS

Covaris AFA technology is in use for numerous Microbiology applications including DNA shearing prior to Next Generation Sequencing (6), Genomic sequencing of Mycobacterium tuberculoisis (7) Human Microbiome Research (8-11), identification of filamentous fungi (12), and liquefication of sputum prior to primary culture for fungi (13).

BIBLIOGRAPHY

1. MALDI Biotyper Standard Operating Procedure Mycobacteria Extraction (MycoEX) Method Bruker protocol rev 3 January 2014

2. truXTRAC MALDI-TOF MS Protocol (p/n 010324), Heat-inactivated Preparation Protocol for Mycobacteria Colony Samples and Analysis on the Bruker MALDI Biotyper® Covaris protocol (p/n 010324, rev C Mar 8, 2016)

3. M-Series User Manual (p/n 010157)

4. Covaris microTUBE Acoustical Cuvette: microTUBE-130 Glass Beads Pre-Slit Screw-Cap (25) (p/n 520199)

5. Covaris Prep Station for eight microTUBE–130 tubes (p/n 500468)

6. www.covarisinc.com/resources/publications

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*Check with Covaris about release date and pricing of the ME220.

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Application Note

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