Four Minute *Mycobacterium* sp. Sample Preparation Process for MALDI-TOF Identification

Philip Onigman¹, Gary Smejkal², Carl Beckett², CoVaris Bill Skea², Srikanth Kakumanu² Jim Laugharn²

¹ Grand Street Consulting, Reading MA USA, ²Covaris Inc, Woburn MA USA

Tel: +1 781-932-3959 • Fax: +1 781-932-8705 Email: info@covarisinc.com Web: www.covarisinc.com

INTRODUCTION AND PURPOSE

Covaris AFA enables an accelerated path to clinical implementation of MALDI-TOF for routine identification of *M. tuberculosis* and NTB.

Many Clinical Microbiology labs delay validation of MALDI-TOF for ID of Mycobacterium spp, due to the time required and inconvenience of sample preparation. We demonstrate an alternative protocol as compared to heating and tube-extraction. Using Covaris Adaptive Focused Acoustics® (AFA®) technology, we report on a safe, rapid, easy-to-use, and robust method that delivers the equivalent or higher MALDI-TOF scores.



Covaris M220 Focused-ultrasonicator and microTUBEs™



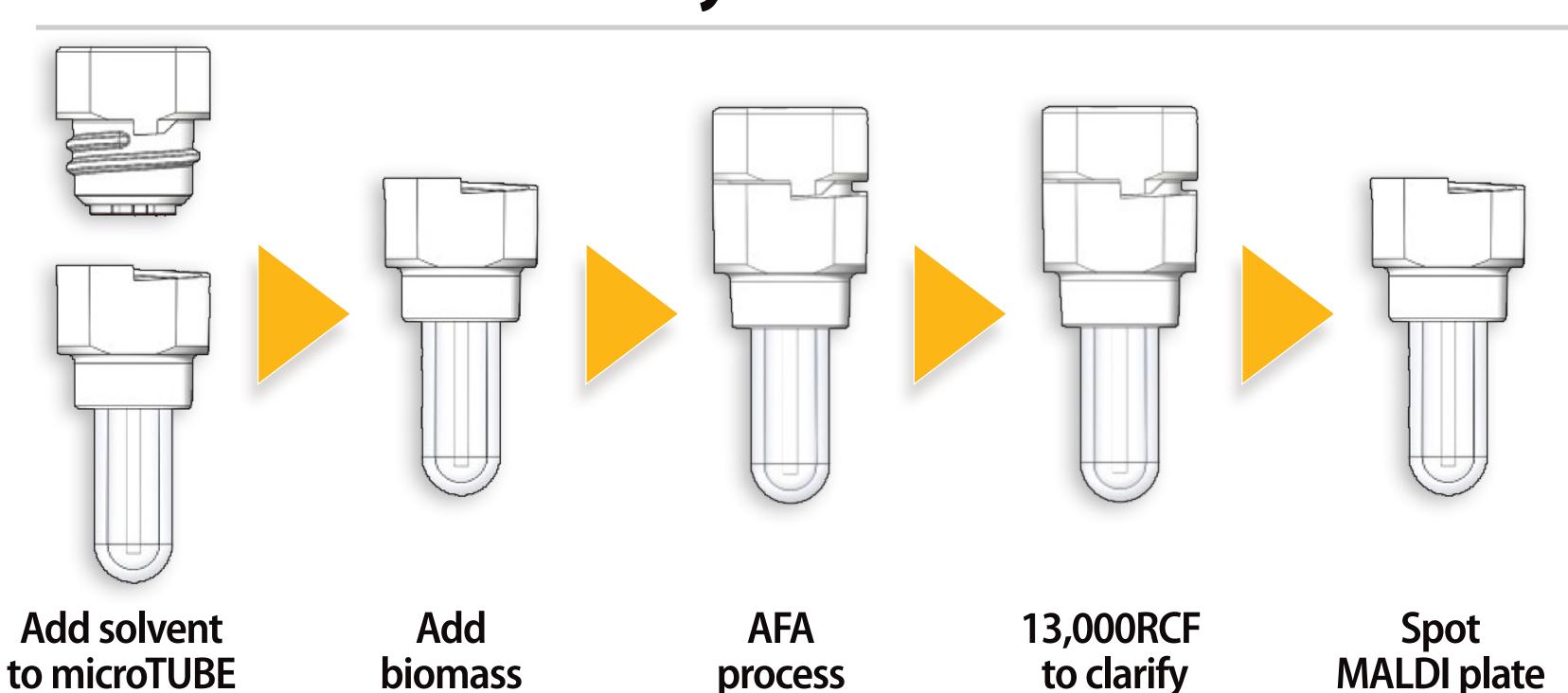
METHODS

M.smegmatis ATCC 19420 was cultured using Middlebrook 7H10 agar plates at 37°C for 48-72 hours. The Bruker MycoEX protocol was used to prepare samples for the Bruker Microflex LT MALDI-TOF with the BDAL and Bruker Mycobacterium database.

M.smegmatis samples were also processed using AFA. The Covaris M220 focused-ultrasonicator delivers highly controlled, isothermal, mechanical energy to each sample. Software controlled the acoustic parameters including peak incidence power, cycles per burst, duty factor, temperature, and time. Covaris microTUBEs (130µL capacity) containing 0.5mm dia beads were used as the process vessel for each sample. A solvent mixture of acetonitrile/formic acid/water (50%:35%:15%) up to 110µL was added. A 1μL loop was used to transfer colonies into each microTUBE with beads and solvent. Covaris microTUBEs are closed vessels that allow non contact processing and prevents aerosols.

Each microTUBE was processed using AFA on the M220 instrument. Temperature was held consistent at 18°C. After the AFA treatment, each microTUBE was centrifuged for 2 minutes at 13,000RCF to clarify. One (1) μL sample volume was applied to the MALDI-TOF target plate. Samples were compared for bio-burden reduction, preparation time, and score consistency. Each sample preparation method was performed in duplicate and five replicates were applied to the MALDI target plate.

AAFA Protocol summary

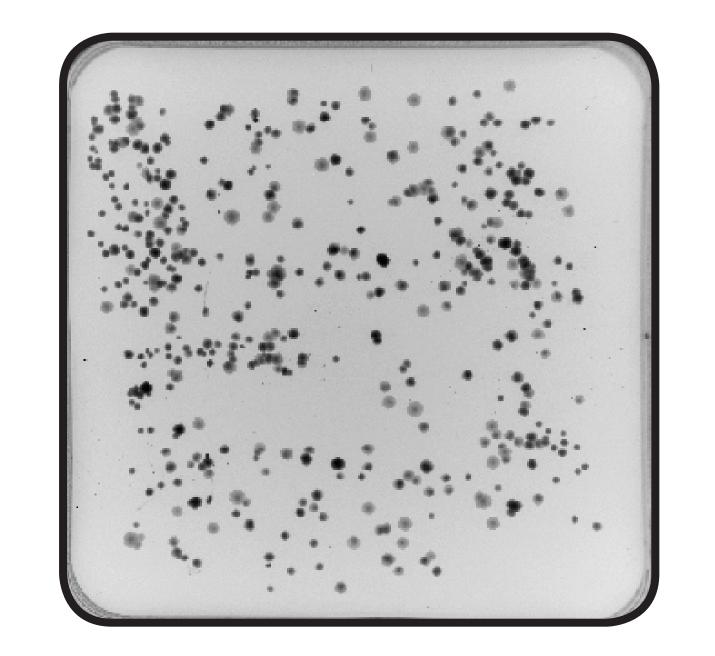


RESULTS

Bioburden Reduction and Handling Safety

The AFA process was equivalent to heating at 100°C for 30 minutes for bioburden reduction. This enables safe handling of AFA-processed samples applied to the MALDI-TOF target plate. The AFA process reduced M. smegmatis challenges of >108 cfu/microTUBE to <10 cfu/microTUBE. This is a significant advance, because heating each sample for 30 minutes prior to MALDI-TOF analysis is considered to be a major inconvenience.

Control (1: 1,000,00)



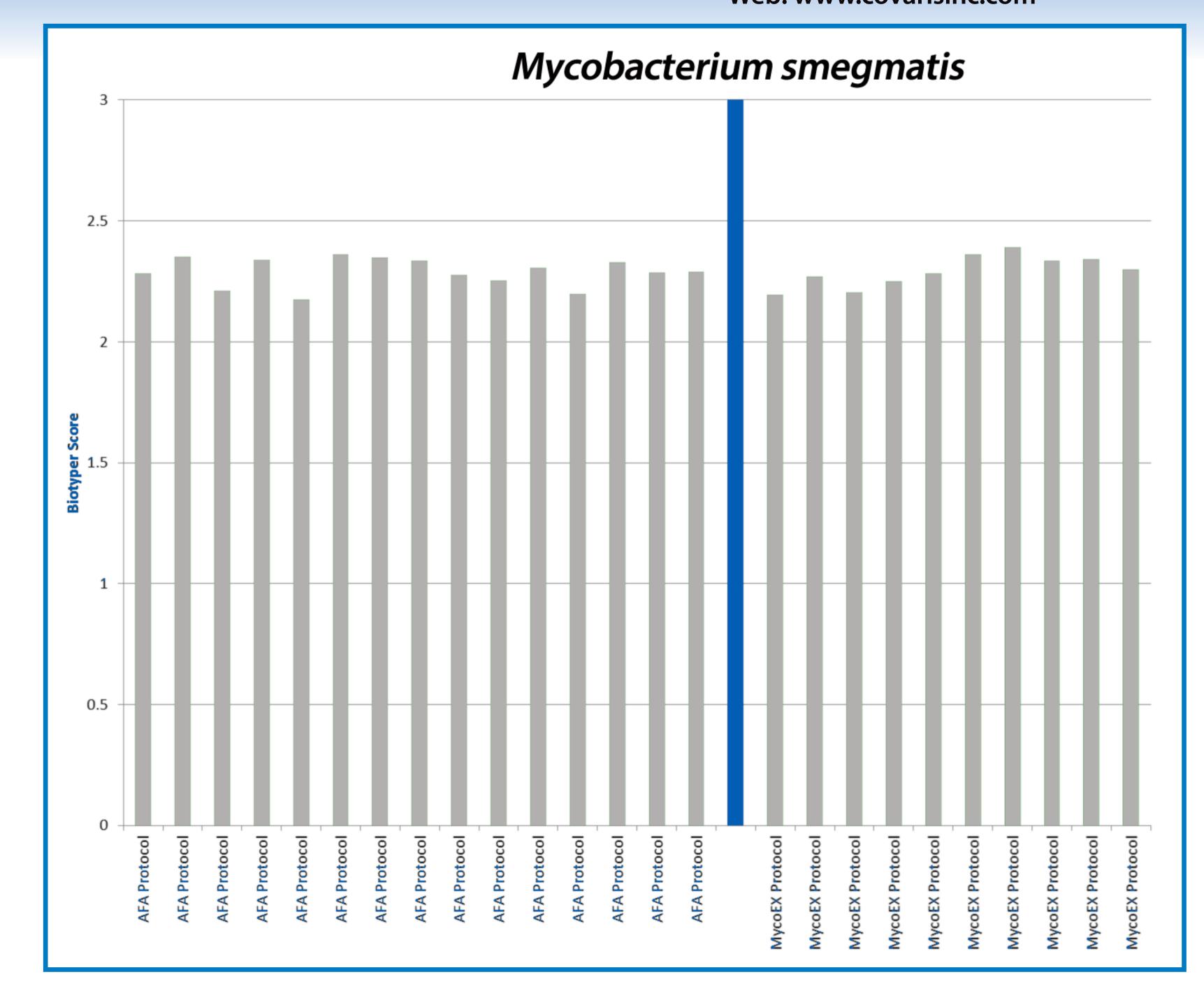
4.4 x 10⁸ cells

AFA - treated

8 - log reduction

AFA Sample Prep MALDI-TOF Scores

Very consistent MALDI-TOF scores > 2.0 resulted in correct identification of genus/species. Centrifugation after the AFA process improved scores. We theorize that removal of insoluble material from the sample prior to application to the MALDI-TOF target plate improves consistency of results.



CONCLUSIONS

AFA provides safe, effective, and rapid sample preparation for identification of Mycobacterium spp by MALDI-TOF. This process eliminates the heat step and consolidates transfer, centrifugation and wash steps into a single reaction vessel. It is easier and more reproducible for laboratory technicians to perform. By reducing send-outs and delays, laboratories can help clinicians manage cases better, further utilize existing capital, and reduce operating costs. The Covaris AFA technology can enable laboratories already using MALDI-TOF to add routine ID of Mycobacterium spp to their clinical service.

Covaris, Inc,14 Gill Street, Unit H, Woburn, MA USA, 781 932 3959 www.covarisinc.com, ponigman@covarisinc.com