Best Practices for Identification of *Mycobacterium* Species Using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

PURPOSE AND BACKGROUND

The ability to control tuberculosis in patients and populations depends on rapid detection and identification of *Mycobacterium tuberculosis* complex (MTBC). This requires a mechanism to accurately identify MTBC and other mycobacteria from primary broth cultures,[1,2] and broth and/or solid media subculture growth.[3–5] Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) is a rapid, sensitive and cost-effective method for organism identification that is routinely used in clinical and public health laboratories. The purpose of this document is to outline best practices for the use of the MALDI-TOF technique as it specifically applies to the identification of *Mycobacterium* species, including both MTBC and non-tuberculous *Mycobacterium* (NTM). This document, structured to mirror a laboratory's own decision and implementation process, will guide readers through the considerations for using the MALDI-TOF system and the necessary steps to implement and maintain testing of MTBC and NTMs using MALDI-TOF. A reader who is considering whether MALDI-TOF is the best fit for their laboratory should review these additional resources.[6–11]

The MALDI-TOF system identifies microbes, usually bacteria or fungal species, through an analytical technique based on the detection and measurement of ionized analytes in the sample.[12,13] When the sample is tested, the MALDI-TOF system generates a spectral profile—referred to as the peptide mass fingerprint (PMF)—which can then be compared to profiles in a database of known PMFs to find a match. Current MALDI-TOF systems generate a report with the ranked matches to library database spectra obtained using an algorithm that indicates the confidence in the MALDI-TOF identification. The identity of a sample can be reliably determined down to the genus level and, in many cases, to the species and strain level.[1–3,5,6]

In the US, there are currently two commercially available MALDI-TOF systems for microbial identification: the MALDI-TOF Biotyper (Bruker Daltonics) and the VITEK MS (bioMérieux) which both have databases that are in vitro diagnostic (IVD) or research use only (RUO). Since there are a large number of published studies of direct comparisons of these two instruments1[4–17] only notable differences related to considerations or implementation of testing for MTBC or NTM will be detailed in this document.

CONSIDERATIONS FOR IMPLEMENTING A MALDI-TOF SYSTEM FOR *MYCOBACTERIUM* IDENTIFICATION

Cost and Justification

The initial purchase of laboratory equipment is always expensive and a MALDI-TOF system is no exception, with instrumentation and software costing approximately $200,000. However, unlike most expensive laboratory instruments, reagent rental agreements are not an option because reagent costs are minimal. Preventive maintenance agreements must also be taken into account as the cost is approximately $20,000/year. Nevertheless, understanding how a new system or workflow has the potential to save staff time, reduce reagent and supply costs, improve the quality of results, and decrease testing turnaround times are all factors that should be examined. This technology can be used by multiple departments or groups within a public health laboratory, which enables cost sharing on the instrument purchase, service agreements, reagents, and potentially staff training and time. Laboratories interested in implementing a MALDI-TOF system can use the information below as justifications. Examples have been provided from public health laboratories that have already purchased/implemented MALDI-TOF systems in their laboratories.

Technology: MALDI-TOF is a relatively new and unique technology that employs an accurate and rapid method for the identification of a wide variety of microbial pathogens, including bacteria, fungi and mycobacteria from culture.

Facilities: The instrument can be placed in a standard Biosafety Level-2 laboratory and generally does not require any specific laboratory facility design (as may be needed with molecular methods).

Expand Identification Services: Extensive spectrum libraries (manufacturer-provided library and software upgrades) allow for an expansion of the number of organisms that can be identified. In some cases, MALDI-TOF provides more specific identifications, which are often not available using traditional testing methods.

Shared Resources: MALDI-TOF has applications for identification of pathogens (i.e., bacterial, fungal) in addition to mycobacteria. Routine costs including service agreements and common reagents, supplies and ancillary equipment, can be shared across departments or groups.

Cost Savings: Labor, consumables and equipment maintenance costs should be compared to the total costs for all current test methods.

- Potential to eliminate or reduce use of other identification or test methods (including method-specific reagents, consumables, and instrument service contracts) if MALDI-TOF is implemented
- Potential to reduce number of staff and time to perform testing (to determine staff time savings)

The following are examples of public health laboratory evaluation of MALDI-TOF systems prior to purchase and implementation:

Example 1: A public health laboratory had saved money in labor and reagent costs for bacterial identification in the bacteriology laboratory through the purchase of a MALDI-TOF system to replace previous bacterial identification methods (e.g., gas chromatography and biochemical tests). The MALDI-TOF system was validated for the identification of mycobacteria to replace a line-probe assay. After one year of routine clinical use in the lab, a cost savings of 96% for reagents and supplies was realized. In addition, a savings of one FTE was realized, and those savings were applied to other components of the identification algorithm. Additionally, the cost to the bacteriology department was reduced, since the equipment and service contract costs could be shared by both bacteriology and mycobacteriology groups.

Example 2: A public health laboratory received funding to purchase a MALDI-TOF system and was asked to evaluate the cost savings over time to justify the purchase. The overall cost to perform MALDI-TOF using the same isolates tested by current testing methods (DNA probes and HPLC) was determined. The number of test runs performed, cost of reagents, chemicals, supplies, consumables, culture media and staff time were considered. Using MALDI-TOF, the estimated cost savings in reagents and supplies was approximately 70% compared to current ID methods. Factoring in service agreement costs, savings were 36%; but since MALDI-TOF has applications that can be utilized across multiple lab areas, these costs could be distributed among them. In this laboratory, the differences in staff time and labor cost were found to be negligible.[18]

Example 3: A public health laboratory is considering using a MALDI-TOF system to replace their current high performance liquid chromatography (HPLC) system, AccuProbe system, and 16s DNA and *rpoB* gene sequencing for the identification of mycobacteria. MALDI-TOF systems can provide identification of a larger number of mycobacteria to the species level than HPLC and the AccuProbe system. Turnaround time would also be decreased. In the bacteriology unit, MALDI-TOF would replace conventional biochemicals, commercial identification system and 16s DNA gene sequencing. The initial capital investment for a MALDI-TOF system is high, but the cost per organism identification is extremely low. In addition, the time to identification will be reduced from 1-10 days to a matter of hours. The elimination of the HPLC system will save $8,000 in preventive maintenance costs, although the MALDI-TOF system annual preventive maintenance cost will be approximately $20,000. Table 1 shows the estimated savings with the switch to a MALDI-TOF system.

Table 1: Example of Financial Justification and Estimated Savings

Application (Test Volume)	Current Method Cost Per Year (Per Test)	MALDI-TOF Cost Per Year (Per Test)*	Annual Savings
Mycobacteria ID by HPLC (2900)	$87,000 ($30)	$6,670 ($2.30)	$80,330
Bacterial ID by API/Biochemical(s) (2500)	$50,000 ($20)	$3,750 ($1.50)	$46,250
Bact/AFB Sequencing ID using 16S/rpoB (200)	$20,000 ($100)	$400 ($2.00)	$19,600
TOTAL ANNUAL SAVINGS			**$146,180**
*Does not include annual preventative maintenance cost of approximately $20,000			

Checklist of Considerations

Safety

CDC states that the incidence of MTBC among persons working with MTBC is 3-5 times greater than laboratory personnel that do not work with MTBC.[19] With an infectious dose of 1-10 organisms and an increased incidence of drug-resistant MTBC, laboratories considering the use of MALDI-TOF for MTBC identification of MTBC must ensure that safety is a top priority.

- Biological risk assessments and validation of inactivation methods (e.g., heat inactivation) should be performed.[20] This assessment is especially important if the instrument will be located in a BSL-2 laboratory.[21] Both commercial systems include inactivation procedures for mycobacteria in their package inserts.
- Each laboratory is responsible for conducting a biological and chemical risk-assessment to evaluate the risks associated with the MALDI-TOF system and with the manipulation of MTBC and NTM. Mitigation steps can then be identified and implemented to minimize the risks.
- An inactivation study must be performed to establish the effectiveness of the culture extraction method in the inactivation of all organisms. The effectiveness of the inactivation method should be assessed periodically as determined by risk assessments, local regulations and/or local practices (See Inactivation and Extraction).

It is essential that laboratories follow the recommendation of the Biosafety in Microbiological and Biomedical Laboratories (BMBL), which states, "BSL-3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of any subspecies of MTBC."[22] Inactivation studies are especially critical for those laboratories with space constraints that necessitate the placement of the MALDI-TOF system in a BSL-2 environment. They must ensure complete inactivation prior to the removal of samples from the BSL-3 laboratory.

Start-up Costs

- Bruker Daltonics MALDI-TOF Biotyper CA System and bioMérieux Vitek® MS instrumentation, software and library packages, installation and training
- Supporting equipment (e.g., heating block, centrifuge, pipettors, etc.)
- Startup supplies (e.g., reagents and chemicals, target slides or plates, consumables, and culture media).

Variable Costs

- Time frame for return on investment acceptable to laboratory administration
- Service contract (after first year) of approximately $20,000 that includes up to two preventive maintenance visits per year and repairs as needed with remote access capability (instrument must be connected to internet)
- Reagents, chemicals, supplies and consumables (e.g., target plates, micro centrifuge tubes, pipette tips, and culture media) needed to perform routine testing based on culture volume
- Staff time to validate new libraries to expand testing capacity across additional laboratory departments or groups: bacteriology, mycology, select agents, etc. Note that the actual library updates are free, but the time needed to validate and implement the updates needs to be considered.

Test Volume and Client Needs

- Consider whether the routine test volume justifies the initial purchase costs (considering current identification method costs compared to MALDI-TOF)
- Evaluate whether the laboratory wants or needs to offer more specific speciation than to the complex level (i.e., MTBC or *Mycobacterium avium* complex (MAC))
- Assess whether the laboratory routinely identifies a large number or many different species of NTMs and whether MALDI-TOF will be helpful in that regard
- Determine whether other laboratory sections (other than Mycobacteriology) would benefit or be interested in using the MALDI-TOF

- Ensure that the libraries available on the platform meet the needs of the laboratory
    - Bacteria, fungi, mycobacteria
    - FDA-cleared or RUO
    - User-created libraries

Staffing

- Identify who will receive initial manufacturer training and perform routine testing
- Identify who will be using the instrument (consider staff across laboratory sections who will be using the MALDI-TOF system)
- Determine the necessary experience and training that is appropriate to perform MALDI-TOF
- Identify who will be responsible for instrument maintenance, quality control, etc.
- Determine potential savings of FTEs or hours required of hands-on testing time (compared to current testing algorithms)

Testing Turnaround Time

- Considering culture, sample preparation and inactivation/extraction requirements
- Address impact of using the MALDI-TOF system in laboratory workflows
- Assess whether scheduling use among laboratory sections would have any impacts

Instrument Placement

- Delivery route for instrument installation and testing workflow
- Electrical requirements
- Temperature/HVAC conditions
- Instrument footprint: ensure adequate space for supporting hardware (keyboard, monitor and printer) and for specimen handling is included
    - bioMérieux is a floor-standing model
    - Bruker is a bench-top model
- Evaluate laboratory space that allows for access and safe and efficient use by all laboratory section staff

IT

- Networking for remote access for data storage and spectrum result analysis
- Interfacing with laboratory information system (LIS) and other instrument systems
- Installation of manufacturer library and software upgrades and manufacturer remote troubleshooting and maintenance
- Create and implement plan for IT security on the MALDI-TOF system

Training

- Ensuring staff are properly trained to manipulate MTBC and NTM safely is critical in reducing the risk of exposure
- More information about working with MTBC can be found in the Laboratory Safety: Work Practices for *Mycobacterium tuberculosis*[23] or other modules in the series "Essentials for the Mycobacteriology Laboratory: Promoting Quality Practice."

CONSIDERATIONS FOR MALDI-TOF VALIDATION

Laboratories should evaluate current testing practices and services, algorithms for identification, and the type and frequency of mycobacteria species routinely identified in their laboratory when determining the MALDI-TOF role. In all situations, primary emphasis should be placed on identifying isolates with clinical and public health significance (e.g., MTBC, *M. avium* complex). Since rapid and accurate identification of MTBC is of primary importance, laboratories should ensure that incorporation of MALDI-TOF in their test algorithm for MTBC improves or enhances current testing for accurate and timely identification of initial cases of MTBC. Priorities for NTM should include species that make up 80-90% of a laboratory's isolates with the remaining "rare" or least-frequently encountered species having the lowest priority. Current practices, resources and cost considerations should guide the decision as to which organisms to validate for identification using MALDI-TOF.[5,7,24]

Initial Validation

The term "validation" is used to describe the process that a manufacturer must follow to set test performance specifications and to describe the process laboratories must follow to determine test performance specifications.[25] A laboratory may need to conduct a validation for a laboratory developed test (LDT) or a modification to an IVD system or procedure. The term "verification" is used to describe the process users (e.g., laboratories) must follow to prove that an individual laboratory can meet manufacturer performance specification under its own conditions.[11] Since MALDI-TOF systems use FDA-cleared or RUO library databases, the extent of the evaluation required for implementation may vary by laboratory. There are many publications that compare the library performance for the identification of mycobacteria.[6,14,26–29] For the purpose of consistency in this document, the term "validation" will be used throughout. Each laboratory should perform a validation or verification as they deem appropriate before implementation.

A validation study to establish performance specifications of the MALDI-TOF systems must be performed prior to reporting test results from patient specimens. CLIA regulations describe the specific requirements for validation.[30] In general, accuracy and precision (i.e., repeatability and reproducibility) are the performance specifications most applicable. Accuracy studies are performed by comparing MALDI-TOF identification results with those obtained using a reference method. CLSI recommends testing a minimum of 30 isolates per organism group (*Mycobacteriaceae*) and comparing to the existing system or to a reference method such as 16S rRNA sequencing and/or previously validated identification method (e.g., line-probe assay).[11] Accuracy studies should also include an assessment of the different culture media used, growth conditions, age of the cultures and extraction protocols used in the test procedure. Precision studies should evaluate reproducibility within runs, between runs, between users/laboratory staff, and over a period of several consecutive days.[11] Since MALDI-TOF is not a quantitative method, validation of linearity, reportable range, and reference range is not applicable. Nevertheless, the confidence of the identification is based on a software system-generated score that is used to define the probability of a correct identification as compared to the reference species in the database. The higher the score, the higher the degree of similarity to a given organism in the database and the greater the confidence of the identification. For example, Bruker provides scores ranging from 3.00 to 0 and interprets the identification as either high confidence, low confidence or no identification possible. Vitek MS provides a percent likelihood score with 99.9% indicating a perfect match with the reference spectrum and an interpretation of a "good ID." "Good ID" scores can range from >60 to 99.8% when a perfect match is not obtained. If a unique spectrum is not recognized, a list of possible organisms is provided with an interpretation of "low discrimination" identification. Laboratories may establish their own minimal scores for acceptable identification and may establish different minimal scores for different organisms. However, if the system provides an FDA-cleared library databases, such as the Vitek MS system, using a score other than that of the manufacturer makes the test a laboratory developed test and therefore, the validation must be more extensive (e.g., using more strains of each species). CLSI recommendations for validation of MALDI-TOF identification systems are available.[11]

Study Design

Acceptability Criteria

Laboratories should establish a reference or "gold standard" method(s) to which MALDI-TOF identifications will be compared. MALDI-TOF will provide a species-level identification for most mycobacteria with some exceptions. The identification resolution of MALDI-TOF may be greater than that provided by routinely validated laboratory methods, which are in many cases only able to provide "group" or "complex"-level identifications. Sequencing methods (e.g., 16S rRNA, *rpoB*, etc.) are generally considered to be the gold standard for bacterial identification, but due to cost and technical limitations, these are not always routinely available. Reference methods such as AccuProbe *Mycobacterium sp*. Identification test (Hologic®), HPLC, and commercial

line-probe methods can be used when sequencing methods are unavailable, but these are not always as discriminatory as MALDI-TOF.

The testing laboratory should establish criteria for an "acceptable" identification using manufacturer guidance, score values and the number of strains of particular species tested. Assessment of MALDI-TOF performance should include determining the number of correct identifications to species/group/complex with reportable scores—per the instrument instructions—for each media type(s) under evaluation (e.g., solid media and broth media) out of the total number of isolates used in the validation (removing isolates for which discrepancies could not be resolved). This will determine the success of the MALDI-TOF platform and protocols under evaluation to provide a usable (reportable) result and correctly identify the isolate. Agreement should then be calculated as the number of correct identifications to species/group/complex per total number of isolates for which there is a reportable score (as per the instrument instructions). MALDI-TOF can be considered validated when it is determined to be equivalent or superior to the existing system. Laboratories should consider at least 90% agreement with current methods as acceptable, with >95% being required for the most commonly isolated species.[31] Additionally, given the serious implications of a misidentification of MTBC, acceptability criteria for MALDI-TOF testing of MTBC isolates should be established as 100%.

Groups/Species That Can Be Identified

In most cases, MALDI-TOF provides a specific genus and species identification. However, in some cases these cannot readily be confirmed because a convenient yet proven supplemental method is unavailable.[11,32] Laboratories should only report identifications to the level of which the validation reference method provides (e.g., MALDI-TOF ID: *M. fortuitum*; reference ID: *M. fortuitum* complex). Reporting at a group, complex or genus level may be acceptable when the healthcare provider does not consider the isolate clinically significant.[33]

Number of Isolates Recommended

Currently, there are > 200 recognized species and subspecies of mycobacteria with standing in nomenclature (e.g., Bacterio.net), and MALDI-TOF databases contain multiple spectra for the majority of these species. Although clinical laboratories routinely identify only a small percentage of these species, those encountered could comprise a wide range of species. Laboratories should determine the distribution and frequencies of mycobacterial species isolated in their laboratory and determine those species that compromise 80-90% of identifications.[31] For these most commonly encountered species, it is suggested that a minimum of 3-5 isolates of each species be tested.[11] Testing a greater number of isolates for the more clinically relevant (e.g., MTBC) and the most frequently isolated species (e.g., *M. avium* complex) should be considered. The majority of test isolates for validation should come from clinical specimens encompassing routine test isolates, but laboratories can include other well-characterized reference strains, including type strains from curated collections (e.g., ATCC, CIP) and isolates from proficiency testing surveys. The number and type of species should be established in consultation with the laboratory director. After initial performance specifications are established, the list of validated species may be expanded. Validation data should be collected on an ongoing basis when uncommon species are isolated and identified. For example, for any species not previously identified in the original validation, a minimum of three of each species must be confirmed by sequencing or other reference methods. Once three or more have been identified and confirmed, confirmation is no longer required and MALDI-TOF results can be reported.

Media

Laboratories should determine which types of media will be used for identification of mycobacteria using MALDI-TOF. Each media type that will be used must be validated. Cultures grown on solid media can be used to assess purity and to provide adequate biomass for testing if needed. Media type (e.g., selective (Middlebrook and Cohn 7H10) vs. non-selective (Lowenstein-Jensen)) generally does not affect identification accuracy, especially when an extraction method is used.[11] Testing broth-based media cultures can decrease testing turnaround time, but any additional proteins from sample inoculation and other present organisms may adversely affect the quality of results.[2,29] Studies have shown some success when using BACTEC™ MGIT™ (Beckton Dickinson) broth media cultures for identification of MTBC using MALDI-TOF.[1,2,4] Laboratories that have validated a MALDI-TOF system have used both solid and broth-based media with differing levels of success. Each laboratory will need to establish a procedure which meets their needs, while optimizing the system and allowing for the best possible quality of results.

Age of Culture

The optimal culture incubation time needed to provide accurate results should be evaluated. One study has shown that culture age generally does not affect spectra quality of a number of mycobacteria species;[34] however, others have demonstrated

that prolonged incubation on solid media does adversely affect the quality of culture identification.[26] Following established procedures and ensuring that cultures are routinely processed and tested at similar time frames will ensure consistency.

Inactivation and Extraction Method

An effective inactivation and extraction method is critical to achieving accurate identification results, while also inactivating the culture. Multiple methods have been described with varying levels of success.[1–4,35] One study found that heat inactivation at temperatures up to 95°C for extended periods of time (up to 30 minutes) using a dry heat block had viable remaining cells, but another successful process where inactivation was achieved was when samples were submerged in a boiling water bath or a forced air oven at 100°C for at least five minutes.[36] A third protocol involves heat inactivation of samples at 80°C for 20 minutes by submersion in a water bath, which demonstrated complete inactivation.[37] The former study did find that effective inactivation was dependent on the internal temperature of the sample reaching the inactivation temperature. Each laboratory should consider temperature, timing, cell density and sample volume when developing an inactivation protocol.[38]

The inactivation and extraction protocols recommended by bioMérieux and Bruker have been shown to be sufficient for inactivating MTBC.[26,38] The procedure recommended by bioMérieux utilizes a combination of lysing bacteria using glass beads and 70% ethanol, whereas the Bruker method inactivates mycobacteria using heat followed by a chemical and glass bead extraction method. These manufacturers' protocols should be performed in the BSL-3 laboratory. For the initial inactivation method validation, 10-12 MTBC strains (clinical and reference) should be evaluated and must include a high concentration of cells (e.g., $>10^6$ or the highest concentration of MTBC used in your laboratory) to thoroughly challenge the inactivation method. This cell suspension should be plated to both liquid and solid media and be incubated for the appropriate amount of time to determine viability of cells (e.g., eight weeks for MTBC).[38] Even after a method has been validated, it is recommended that laboratories continually monitor the efficacy of their inactivation procedure as determined by their own risk assessment.[39] As an example, one public health laboratory used 10 clinical and one reference strain and monitors inactivation monthly using material from three confirmed MTBC cultures.

Integrating MALDI-TOF into a Laboratory Diagnostic Algorithm

Some laboratories have experienced difficulties when attempting to identify mycobacteria directly from primary broth cultures inoculated with clinical specimens.[1,2,40] Other institutions have developed and optimized alternative extraction methods designed to more effectively identify mycobacteria directly from primary broth cultures.[1,2,4] If a laboratory determines that the primary culture identification turnaround time of MALDI-TOF is inadequate, then alternative methods can be used in combination with MALDI-TOF to create a more comprehensive and efficient identification algorithm.

MALDI-TOF can reliably identify many mycobacteria species from primary broth and solid cultures, though laboratories may choose to subculture positive broth cultures to both solid and broth media. If using liquid broth culture for MALDI-TOF, consideration should also be given to sub-culturing from broth onto solid media to ensure that low level secondary mycobacteria are not missed and to be able to confirm colony characteristics are consistent with MALDI-TOF ID. Rapid detection assays such as PCR-based tests, rapid antigen detection and hybridization assays can be performed on primary broth cultures to identify MTBC, and may be the preferred method for cultures that exhibit features indicating possible MTBC (e.g., cording observed in AFB smear from culture).

Pure mycobacterial cultures that cannot be identified using the testing methods discussed above or provide identifications requiring further validation may be tested using alternative identification methods, such as gene sequencing or submission to reference laboratories.

CONSIDERATIONS FOR REPORTING RESULTS

Results Interpretation and Supplemental Testing

Results generated from the MALDI-TOF should be reported in accordance with CLIA guidelines. After the assay has been validated, results should be reported as indicated by the validation (either to the species or group/complex level). For example, results may be reported as MTBC-identified or *M. avium*-identified. While it is not required that the testing method be specified on the report, in accordance with CLIA regulation 42 CFR 493.1291,30 "the laboratory must, upon request, make available to clients a list of test methods employed by the laboratory and, as applicable, the performance specifications established or verified as specified in 493.1253." Section 493.1291(c)(4)30 does stipulate that any test which has not been FDA-cleared or FDA-approved, including those not subject to FDA clearance or approval, methods developed in house, standardized methods

and FDA-approved procedures which have been modified by the user must include the disclaimer statement "The performance characteristics of this test were determined by (Laboratory Name). It has not been cleared or approved by the US Food and Drug Administration."

ONGOING CONSIDERATIONS

Adding New Organisms to the Library

Identification of bacterial organisms using MALDI-TOF is limited by the number and quality of spectra in the database. To enhance identification, library entries can be added to a local database for species more frequently encountered in a specific laboratory. Literature review highlights the many benefits of augmenting the manufacturer's library with additional reference spectra, such as including species not represented in the manufacturer's database, enriching the depth of coverage in the manufacturer's database, or incorporating clinical strains whose spectral profiles are distinct from type strains found in the database.[41,42] Previous studies have reported enhanced libraries are required for identification for several classes of bacteria including *Mycobacterium spp.*, *Nocardia spp.*, and aerobic actinomycetes.[26,43] Indeed, a recent study reported that with a custom library 88% of mycobacterial species could be identified with a score of 2.0, the acceptable score at the time.[7] When they lowered the score to 1.7 they achieved 91% identification. While this study used only isolates from solid media, the agreement was quite good and there were no misidentifications.[7] When creating a custom library, important considerations should be made including selection and identity confirmation of bacterial strains to be added, as well as spectral quality, and the number of spectra required for validation.[44] Strains added to a custom library should be verified with several testing methods, as addition of an incorrect organism during this stage will lead to inaccurate identification in the future. Organism identification for mycobacteria should be confirmed using DNA sequence analysis using targets such as 16S rRNA, *rpoB*, *hsp65*, and *gyrB* genes, and identifications and should have a percent identification greater than 99% to only one organism (unless being entered into the library as a complex). When creating a new library, each entry should be created using 20-36 spectra collected from 9-12 distinct spots on the target plate. All spectra should be analyzed using the manufacturer's recommended criteria and the new entry should be run against all the other spectral profiles in all libraries to ensure the identification score is acceptable and specific for that species of mycobacteria.[7] It is important to note that multiple strains of the same species may be added to the library to enhance identification of the organism. In addition, strains added to the custom library should be prepared following the same extraction methods and growth conditions as those utilized in the laboratory to allow for optimal identification of unknowns. Validation of newly added strains should be conducted following the same guidance discussed previously.

Verification of New Versions of Commercial Libraries

When installing new versions of commercially-available libraries, users may wish to do a limited verification study, primarily focused on organisms that have been added or changed. The manufacturer should supply information detailing changes to the library associated with the new version, which should include details on any new organism entries and other significant changes to the database. Based on this information, a verification study can be designed and performed to test the system's ability to accurately identify the new organisms. If the organisms added to the database are rarely isolated, it may not be feasible to perform a verification study. If isolated, these species will need to be confirmed by the laboratory's gold standard" testing method.[11]

An alternate way to validate new library versions is to reanalyze spectra from previous MALDI-TOF runs. One can select and compile a defined number of spectra files, representing the most commonly identified organisms. For example, 5-20 spectra each of 10-20 different species could be compiled and analyzed using the previous library version, and again after the new update has been installed. Comparing these results side-by-side will help insure that the updated system continues to meet the laboratory's requirements. This will also give an indication of how the library update may impact identification of the laboratory's most commonly isolated organisms.

Quality Control and Quality Assurance

Equipment Maintenance

Most of the routine maintenance of the MALDI-TOF system is performed by the manufacturer's field service representatives. Some manufacturers can monitor and fine tune the instrument performance remotely and notify the user when adjustments are made. This can include hardware adjustments, software updates and troubleshooting. The laboratory should follow the manufacturer's recommendations for routine maintenance that is required to be performed by the user to keep the instrument

performing reliably. All maintenance and service on the equipment should be documented and retained in accordance with each laboratory's policy.

Troubleshooting

A well-trained and experienced laboratory staff is required for effective MALDI-TOF troubleshooting. When troubleshooting, other characteristics of the organisms such as colony morphology, growth rate, results of biochemical tests performed, and antibiotic susceptibility test results should be taken into account. Table 2 describes possible causes for the absence of a spectrum, and poor quality or unusual spectra. If an instrument problem is suspected, the manufacturer should be contacted. The technical service representative may be able to remotely access the MALDI-TOF system to diagnose and correct the issue.

Table 2. Troubleshooting for Absent or Unexpected ID: Possible Causes, Stratified by Spectrum Quality

Poor Quality or No Mass Spectrum Generated – No ID Obtained
• Matrix was not added before analysis. • Too much or too little biomass was used in analysis. • Biomass and/or matrix do not cover the entire target spot. • Isolate is mucoid; too much capsular material was transferred to target. • Organism type is not responsive to the direct colony transfer technique; extraction is needed. • Organism biomass is contaminated with agar or primary specimen. • Culture is mixed (organism not properly isolated). • Reagents have expired/evaporated/crystallized or were incorrectly prepared. • Organism is too old or too young. • Organism was refrigerated before analysis. • Scratches or dents are on the target slide or plate. • Laser intensity is inadequate. • Detector needs maintenance or replacement. • Uneven target spot application.
Good Quality Mass Spectrum Generated – Unexpected ID, Split ID, or No ID Obtained
• The sample was transferred to the wrong spot on the target slide or plate (inaccurate sample tracking). • Sample cross-contamination is present (may occur from sloppy spotting, using a single pipette to apply the matrix to multiple spots, or from inadequate cleaning of reusable target slides or plates). • The culture is mixed (microorganism not properly isolated before analysis). • Reagents are contaminated with microorganisms. • Organism is not represented in the database.

Abbreviation: ID, identification(s).

Reprinted with permission from Clinical and Laboratory Standards Institute from: CLSI. Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. 1st ed. CLSI guideline M58. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.11

REFERENCES

1. Huang T-S, Lee C-C, Tu H-Z, Lee SS-J. Rapid identification of mycobacteria from positive MGIT broths of primary cultures by MALDI-TOF mass spectrometry. PLOS ONE. 2018 Feb 2;13(2):e0192291.

2. Miller E, Cantrell C, Beard M, Derylak A, Babady NE, McMillen T, et al. Performance of Vitek MS v3.0 for Identification of *Mycobacterium* Species from Patient Samples by Use of Automated Liquid Medium Systems. Forbes BA, editor. J Clin Microbiol. 2018 Jun 6;56(8). Available from: http://jcm.asm.org/lookup/doi/10.1128/JCM.00219-18

3. Rodriguez-Temporal D, Perez-Risco D, Struzka EA, Mas M, Alcaide F. Evaluation of Two Protein Extraction Protocols Based on Freezing and Mechanical Disruption for Identifying Nontuberculous Mycobacteria by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry from Liquid and Solid Cultures. Forbes BA, editor. J Clin Microbiol. 2018 Apr 1;56(4):e01548-17.

4. Morales MPF, Lim CK, Shephard L, Weldhagen GF. Mycobacterial inactivation protein extraction protocol for matrix-assisted laser desorption ionization time-of-flight characterization of clinical isolates. Int J Mycobacteriology. 2018 Sep;7(3):217–21.

5. Body BA, Beard M, slechta ES, Hanson KE, Barker AP, Babady NE, et al. Evaluation of the Vitek MS v3.0 Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Identification of *Mycobacterium* and *Nocardia* Species. J Clin Microbiol. 2018 May 25;56(6):e00237-18.

6. Wilen CB, McMullen AR, Burnham C-AD. Comparison of Sample Preparation Methods, Instrumentation Platforms, and Contemporary Commercial Databases for Identification of Clinically Relevant Mycobacteria by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. J Clin Microbiol. 2015 Jul 1;53(7):2308–15.

7. Buckwalter SP, Olson SL, Connelly BJ, Lucas BC, Rodning AA, Walchak RC, et al. Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of *Mycobacterium* species, *Nocardia* species, and Other Aerobic Actinomycetes. Forbes BA, editor. J Clin Microbiol. 2016 Feb 1;54(2):376.

8. van Belkum A, Welker M, Pincus D, Charrier JP, Girard V. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry in Clinical Microbiology: What Are the Current Issues? Ann Lab Med. 2017 Nov;37(6):475–83.

9. Cao Y, Wang L, Ma P, Fan W, Gu B, Ju S. Accuracy of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Mycobacteria: a systematic review and meta-analysis. Sci Rep. 2018 Mar 7;8(1):4131.

10. Alcaide F, Amlerová J, Bou G, Ceyssens PJ, Coll P, Corcoran D, et al. How to: identify non-tuberculous *Mycobacterium* species using MALDI-TOF mass spectrometry. Clin Microbiol Infect. 2018 Jun 1;24(6):599–603.

11. Clinical Laboratory Standards Institute (CLSI). M58 Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. 1st Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2017. 92 p. Available from: https://clsi.org/standards/products/microbiology/documents/m58/

12. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol. 2015 Aug 5;6:791.

13. Cobo F. Application of MALDI-TOF Mass Spectrometry in Clinical Virology: A Review. Open Virol J. 2013 Sep;7:84–90.

14. Lévesque S, Dufresne PJ, Soualhine H, Domingo M-C, Bekal S, Lefebvre B, et al. A Side by Side Comparison of Bruker Biotyper and VITEK MS: Utility of MALDI-TOF MS Technology for Microorganism Identification in a Public Health Reference Laboratory. PLoS ONE. 2015 Dec 10;10(12):e0144878.

15. Marko DC, Saffert RT, Cunningham SA, Hyman J, Walsh J, Arbefeville S, et al. Evaluation of the Bruker Biotyper and Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems for Identification of Nonfermenting Gram-Negative Bacilli Isolated from Cultures from Cystic Fibrosis Patients. J Clin Microbiol. 2012 Jun 1;50(6):2034–9.

16. Ferrand J, Hochard H, Girard V, Aissa N, Bogard B, Alauzet C, et al. Comparison of Vitek MS and MALDI Biotyper for Identification of Actinomycetaceae of Clinical Importance. Munson E, editor. J Clin Microbiol. 2016 Mar 1;54(3):782.

17. Jamal W, Albert JM, Rotimi VO. Real-time comparative evaluation of bioMerieux VITEK MS versus Bruker Microflex MS, two matrix-assisted laser desorption-ionization time-of-flight mass spectrometry systems, for identification of clinically significant bacteria. BMC Microbiol. 2014 Nov 30;14.

18. Tran A, Alby K, Kerr A, Jones M, Gilligan PH. Cost Savings Realized by Implementation of Routine Microbiological Identification by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. Am Soc Microbology. 2015 May 15;53:2473–2479.

19. Carter JR. Goals for Working Safely With Mycobacterium tuberculosis in Clinical, Public Health, and Research Laboratories. Fed Regist 23066. 1997 Apr 21;62(81). Available from: https://www.govinfo.gov/content/pkg/FR-1997-04-28/pdf/97-10879.pdf

20. Rudrik JT, Soehnlen MK, Perry MJ, Sullivan MM, Reiter-Kintz W, Lee PA, et al. Safety and Accuracy of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Highly Pathogenic Organisms. J Clin Microbiol. 2017 Dec;55(12):3513–29.

21. Risk Assessment Best Practices. Silver Spring, MD: Association of Public Health Laboratories; 2016 May. Available from: https://www.aphl.org/programs/preparedness/Documents/APHL%20Risk%20Assessment%20Best%20Practices%20and%20Examples.pdf

22. Biosafety in Microbiological and Biomedical Laboratories. Department of Health and Human Services; 2009. Available from: https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF

23. Association of Public Health Laboratories. Laboratory Safety: Work Practices for Mycobacterium tuberculosis in the Essentials for the Mycobacteriology Laboratory: Promoting Quality Practices. Silver Spring, MD. Available from: https://www.aphl.org/programs/infectious_disease/tuberculosis/Pages/Training-Modules.aspx

24. Rodríguez-Sánchez B, Ruiz-Serrano MJ, Marín M, López Roa P, Rodríguez-Créixems M, Bouza E. Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Nontuberculous Mycobacteria from Clinical Isolates. J Clin Microbiol. 2015 Aug;53(8):2737–40.

25. Clinical Laboratory Standards Institute (CLSI). MM 17 Validation and Verification of Multiplex Nucleic Acid Assays, 2nd Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.118 p. Available from: https://clsi.org/standards/products/molecular-methods/documents/mm17/

26. Mather CA, Rivera SF, Butler-Wu SM. Comparison of the Bruker Biotyper and Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems for Identification of Mycobacteria Using Simplified Protein Extraction Protocols. Dunne WM, editor. J Clin Microbiol. 2014 Jan 1;52(1):130.

27. Epperson LE, Timke M, Hasan NA, Godo P, Durbin D, Helstrom NK, et al. Evaluation of a Novel MALDI Biotyper Algorithm to Distinguish *Mycobacterium intracellulare* From *Mycobacterium chimaera*. Front Microbiol. 2018 Dec 18;9:3140–3140.

28. Rodriguez-Temporal D, Perez-Risco D, Struzka EA, Mas M, Alcaide F. Impact of updating the MALDI-TOF MS database on the identification of nontuberculous mycobacteria. J Mass Spectrom. 2017 Sep;52(9):597–602.

29. Leyer C, Gregorowicz G, Mougari F, Raskine L, Cambau E, de Briel D. Comparison of Saramis 4.12 and IVD 3.0 Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Mycobacteria from Solid and Liquid Culture Media. J Clin Microbiol. 2017 Jul;55(7):2045–54.

30. Centers for Medicare and Medicaid Services. CFR Standard: Establishment and verification of performance specifications. Sect. 493.1253, Title 42 Aug 22, 2003. Available from: https://www.govinfo.gov/app/details/CFR-2010-title42-vol5/CFR-2010-title42-vol5-sec493-1253

31. Clinical Laboratory Standards Institute (CLSI). M52 Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2015. 92 p. Available from: https://clsi.org/standards/products/microbiology/documents/m52/

32. Turenne CY. Nontuberculous mycobacteria: Insights on taxonomy and evolution. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2019 Aug;72:159–68.

33. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007 Feb 15;175(4):367–416.

34. Dunne WM, Doing K, Miller E, Miller E, Moreno E, Baghli M, et al. Rapid Inactivation of *Mycobacterium* and *Nocardia* Species before Identification Using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. J Clin Microbiol. 2014 Oct 1;52(10):3654–9.

35. Niitsuma K, Koshiba S, Saitou M, Suzuki T, Chikamatsu K, Takagi A, et al. Use of Ultrasonication as a Rapid Pretreatment Method for MALDI-TOF MS of Mycobacterial Samples. Mycobact Dis. 2018; 08(02). Available from: https://www.omicsonline.org/open-access/use-of-ultrasonication-as-a-rapid-pretreatment-method-for-malditof-ms-of-mycobacterial-samples-2161-1068-1000260-101114.html

36. Zwadyk P, Down JA, Myers N, Dey MS. Rendering of mycobacteria safe for molecular diagnostic studies and development of a lysis method for strand displacement amplification and PCR. J Clin Microbiol. 1994 Sep;32(9):2140–6.

37. Doig C, Seagar AL, Watt B, Forbes KJ. The efficacy of the heat killing of *Mycobacterium tuberculosis*. J Clin Pathol. 2002 Oct;55(10):778–9.

38. Blackwood KS, Burdz TV, Turenne CY, Sharma MK, Kabani AM, Wolfe JN. Viability testing of material derived from *Mycobacterium tuberculosis* prior to removal from a Containment Level-III Laboratory as part of a Laboratory Risk Assessment Program. BMC Infect Dis. 2005 Jan 24;5(1):4.

39. Association of Public Health Laboratories. Molecular Detection and Identification of Mycobacteria in the Essentials for the Mycobacteriology Laboratory: Promoting Quality Practices. Silver Spring, MD. Available from: https://www.aphl.org/programs/infectious_disease/tuberculosis/Pages/Training-Modules.aspx

40. van Eck K, Faro D, Wattenberg M, de Jong A, Kuipers S, van Ingen J. Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Fails To Identify Nontuberculous Mycobacteria from Primary Cultures of Respiratory Samples. McAdam AJ, editor. J Clin Microbiol. 2016 Jul 1;54(7):1915–7.

41. Nomura F. Proteome-based bacterial identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS): A revolutionary shift in clinical diagnostic microbiology. Biochim Biophys Acta. 2015 Jun;1854(6):528–37.

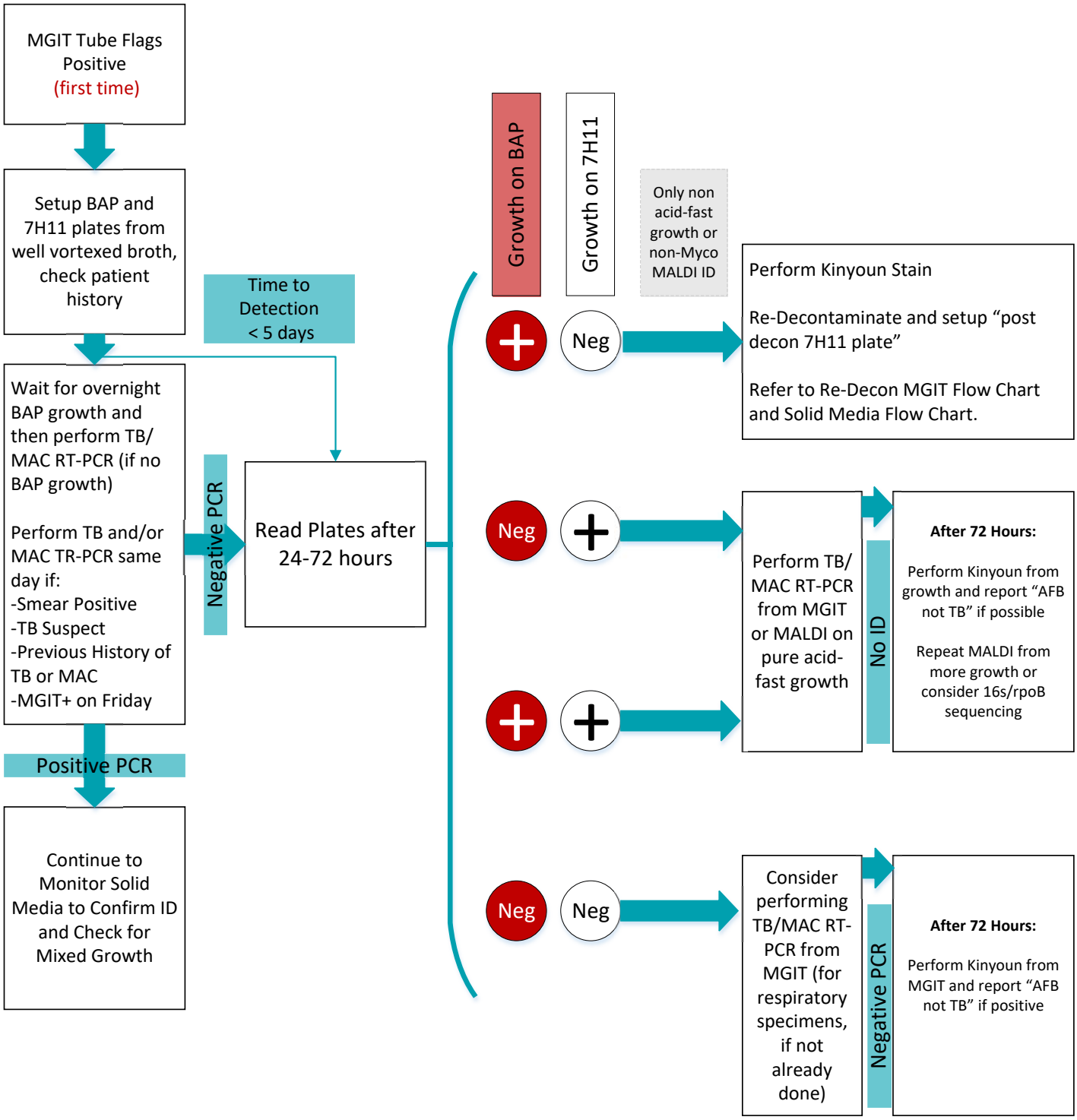
42. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem. 2015 Jan;61(1):100–11.

43. Saleeb PG, Drake SK, Murray PR, Zelazny AM. Identification of mycobacteria in solid-culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2011 May;49(5):1790–4.

44. Clinical Laboratory Evaluation Program. Guidelines for Submission of Validation Packages for Approval of MALDI-TOF Mass Spectrometry (MALDI-TOF MS) for Bacterial, Yeast and Mold Identification. Albany, NY: Wadsworth Center, New York State Department of Health; 2015 Jul. Available from: https://www.wadsworth.org/sites/default/files/WebDoc/MALDI-TOF.pdf

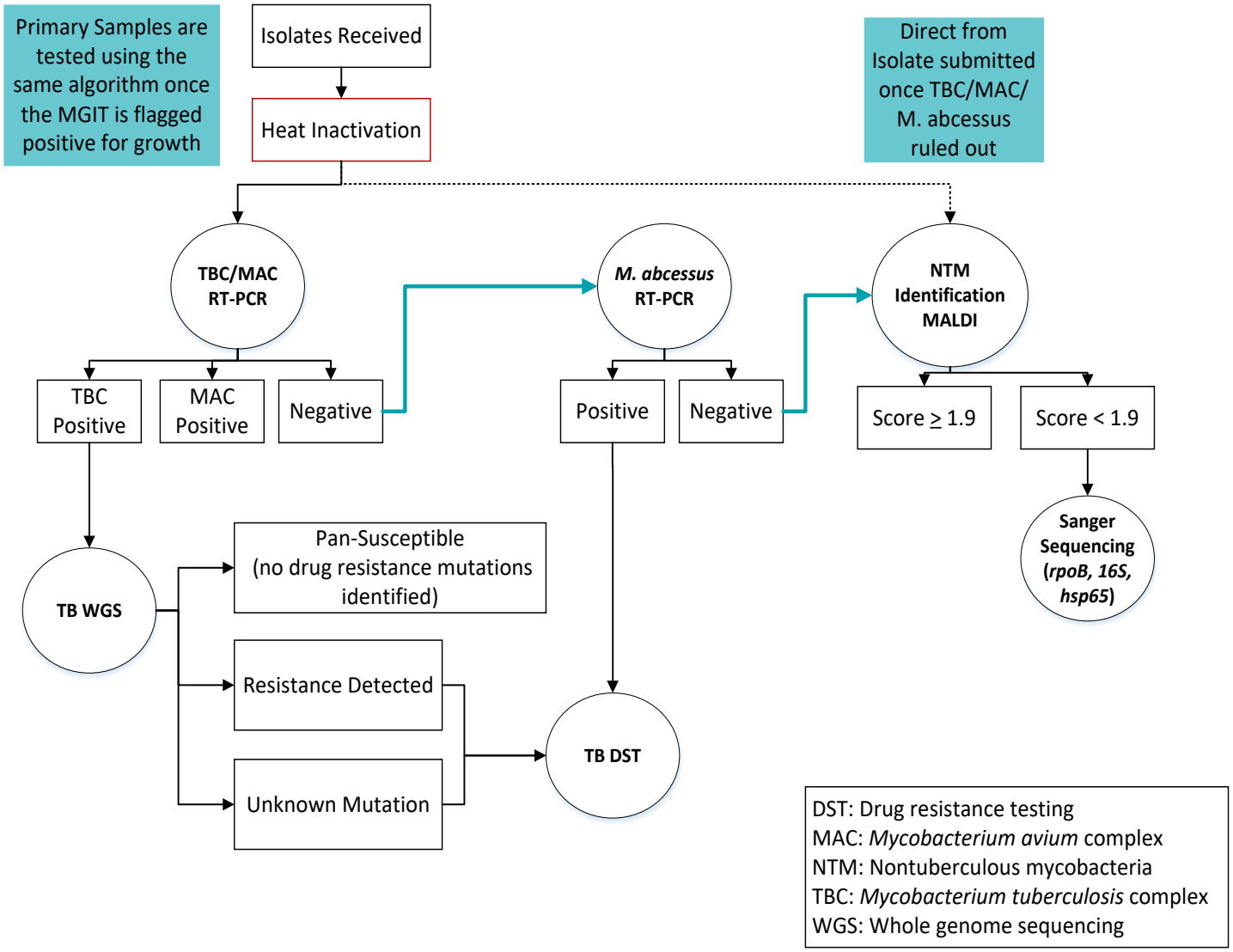
APPENDIX A: EXAMPLE WORK FLOWS

Example 1



APPENDIX A: EXAMPLE WORK FLOWS

Example 2



ACKNOWLEDGEMENTS

The idea for this document was developed by the APHL TB MALDI-TOF Users Group and was made possible through the TB MALDI-TOF Best Practices Writing Group and the APHL TB Subcommittee, with significant input and contributions from the following individuals:

Gary Budnick, Dr. Katherine A. Kelley Public Health Laboratory (CT)

Donald Busalacchi, Wisconsin State Laboratory of Hygiene

Drew Francis, (ASCP)M, Arizona State Laboratory Services

Jessica Gentry, Indiana State Department of Health Laboratory Services

Frances Jamieson, MD, FRCPC, Public Health Ontario

Latricia Lewis, Houston Health Department, Bureau of Laboratory Services

Kara Mitchell, PhD, New York State Department of Health, Wadsworth Center

Jafar Razeq, PhD, HCLD (ABB), Dr. Katherine A. Kelley Public Health Laboratory (CT)

Tracy Stiles, (ASCP)M, William A. Hinton State Laboratory Institute (MA)

David M. Warshauer, PhD, D(ABMM), Wisconsin State Laboratory of Hygiene

Stacy White, PhD, Arizona State Laboratory Services

Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

This project was 100% funded with federal funds from a federal program of $905,171. This publication was supported by Cooperative Agreement #5 NU60OE000103 from the US Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC.

8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Phone: 240.485.2745
Fax: 240.485.2700
www.aphl.org