UnityPoint Health METHODIST MICROBIOLOGY LABORATORY	Page 1 of 15	Section: UPM MICRO	Policy #: 11.015
	Approved by: see signature block at end of document		Date: 03/27/18 Review by: 03/27/20
	Supersedes: 3/27/18 Date Revised:		
	Primary Responsible Parties: Marsha Bishoff Secondary Responsible Parties: Audrey Davis		
	CAP Standard:		
SUBJECT: BRUNKER MALDI BIOTYPER CA			

PRINCIPLE

The MALDI Biotype CA System uses mass spectrometry technology for the identification of bacteria isolated from clinical specimens.

An isolated colony from an overnight culture is transferred to a selected position on a sample target. After inoculation, the target is air dried and matrix is added. The Standard Solvent in the matrix solution extracts proteins (mainly ribosomal proteins, which are present in high concentration) from the bacteria. When dried matrix crystallizes, the inoculated target is ready to be analyzed on the MALDI Biotyper CA System.

Samples are analyzed using MALDI (matrix-assisted laser desorption/ionization) TOF (time-of-flight) mass spectrometry. First, the MALDI process transforms the proteins and peptides from the isolated bacteria into positively charged ions. This is achieved by irradiating the matrix-sample composite with a UV laser. The matrix absorbs laser energy and transfers protons to the intact proteins or peptides in the gas phase. Then, these ions are electrostatically accelerated and arrive in the flight tube at a mass-dependent speed. Because different proteins/peptides have different masses, ions arrive at the detector at different times (time of flight). The MALDI Biotyper CA System measures the time (in the nanosecond range) between pulsed acceleration and the corresponding detector signal of the ions, and the time is converted into an exact molecular mass.

The highly abundant microbial ribosomal proteins result in a mass spectrum with a characteristic mass and intensity distribution pattern. This pattern is species-specific for many bacteria and can be used as a “molecular fingerprint” to identify a test organism. The acquired mass spectra are electronically transformed into peak lists. Peak lists are compared to all patterns in the reference library

CLINICAL SIGNIFICANCE

The MALDI Biotyper CA System is a qualitative *in vitro* diagnostic device indicated for use in conjunction with other clinical and laboratory findings to aid in the diagnosis of bacterial and yeast infections.

A guiding principle of the clinical microbiology laboratory is to provide patient test results as quickly as possible while maintaining accuracy and quality. Therefore, advancements in analytical technology that not only improve accuracy and quality, but also decrease the length of time from sample collection to results are highly desirable. In the clinical microbiology laboratory where many traditional culture-based methods take days to yield results, providing diagnostic information in a matter of hours using mass spectrometry (MS) and molecular techniques holds the potential for vast improvements in patient care.

One of the major drivers today for providing rapid microbiology test results is the practice of antimicrobial stewardship, which aims to reduce the amount of unnecessary antibiotics administered before laboratories definitively identify the type of bacterial infection. Combining laboratory and pharmacy practices in a stewardship program has several advantages, including minimizing patient exposure to IV antibiotics, decreasing the possibility of microorganisms developing antibiotic resistance, and reducing costs associated with prolonged hospital stays.

POLICY SCOPE:

The scope of this policy applies to all Laboratory staff that prepares or performs testing on laboratory specimens at UnityPoint Methodist.

SPECIMEN

Biosafety Level 2

Specimen Collection and Preparation

Appropriate specimens should be collected, transported, and placed on primary isolation media according to procedures recommended in the *Manual of Clinical Microbiology*.[1] Test organism samples intended to be analyzed in the MALDI Biotyper CA System must be prepared at room temperature (+20°C/+68°F-+25°C/+77°F)

Culture requirements

For measurement in the MALDI Biotyper CA System, isolates must be tested from a fresh culture – recommended cultivation of 18-48 h - or 12 hours post incubation at room temperature.

Identification must be performed on a single isolated bacterial colony. Mixed samples may result in erroneous identification.

Specific requirements

- *Bordetella*: Incubation on BG agar should not be longer than 24h (+12h storage at RT).
- *Campylobacter*: Incubation can be prolonged to 72h (+12h storage at RT).
- *Streptococcus pneumoniae*: Incubation should not be longer than 24h (+12h storage at RT) due to possible autolysis.
- *Neisseria*: Incubation on Modified Thayer-Martin Agar should not be longer than 24h (+12h storage at RT).

Approved Media for Culture

Columbia Blood w/5% Sheep Blood	Brucella w/5% Sheep Blood	C difficile w/7% Sheep Blood
Trypticase Soy w/5% Sheep Blood	CDC Anaerobe w/5% Sheep Blood	Sabouraud Dextrose
Chocolate	CDC Anaerobe PEA w/5% Sheep Blood	Brain Heart Infusion
MacConkey	CDC Anaerobe LKV	Campylobacter w/10% Sheep Blood
Columbia CNA w/5% Sheep Blood	Modified Thayer-Martin	Bordet Gengou w/15% Sheep Blood

*****Refer to System Package Insert for more acceptable media

Determination of Specimen Quality:

- Do not test colonies growing on culture media other than that previously specified.
- Do not test colonies growing on culture media which appears damaged by heat (dehydration) – subculture the isolate to a fresh sheep blood agar plate or chocolate agar plate.
- Do not test colonies growing on culture media which contains excessive moisture – subculture the isolate to a fresh sheep blood or chocolate agar plate.
- Do not test colonies growing in the presence of swarming Proteus species unless the colony is sufficiently separated from the swarm – subculture the isolate to a fresh sheep blood or chocolate agar plate to ensure purity.

REAGENTS

- US IVD 48 Spot Target
- US IVD BTS (Bacterial Test Standard)
- US IVD HCCA Matrix
- Standard Solvent (acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%) – Expires 6 months after opening.*
- Acetonitrile*
- HPLC- grade water
- Formic acid (FA)*
- absolute Ethanol (EtOH) *
- Trifluoroacetic acid (TFA) *
- Sterile Colony Transfer Device
- Eppendorf pipette tips 0.5-20 µL, 2-200 µL, 50-1000 µL
- Suitable pipettes for volumes from 1 µL to 1000 µL
- Eppendorf plastic tubes, 1.5 mL
- Screw-cap micro tubes and screw caps
- Bench-top microcentrifuge capable of 13,000 to 15,000 rpm
- Vortex mixer
- Standard laboratory equipment

*These chemicals are stored at room temperature in a chemical safety cabinet.

For best results, preparation of all solutions, Standard Solvent, and the entire sample preparation process including drying steps must be performed under controlled room temperature.

US IVD HCCA Matrix Preparation

US IVD HCCA Matrix is shipped at ambient temperature. Store in the refrigerator at 2°C to 8°C (36°F to 46°F) until preparation. Do not use after expiration date.

1. Add 250 µL Standard Solvent into one tube of US IVD HCCA portioned [final concentration: 10 mg HCCA/mL].

2. Use the recommended commercially available Standard Solvent or mix 475 µL HPLC grade water, 25 µL trifluoroacetic acid, and 500 µL acetonitrile in a 1.5 mL Eppendorf tube to produce 1 mL Standard Solvent.
3. Shake the screw-cap tube and use a vortex mixer to completely dissolve US IVD HCCA portioned at room temperature. Make sure all crystals are completely dissolved.
4. Shake the contents down. The clear US IVD HCCA portioned solution is now ready for use.

Note: Reconstituted US IVD HCCA portioned solution is stable at controlled room temperature (20-25°C / 68-77°F) for up to one week.

US IVD BTS Preparation

US IVD BTS is shipped at ambient temperature but must be stored at -18°C/0°F or below immediately on arrival. The expiry date on the package is valid for the enclosed US IVD BTS when stored at -18°C/0°F or below on arrival.

1. Bring US IVD BTS to room temperature for a minimum of five (5) minutes before use.
2. Add 50 µL of Standard Solvent and dissolve by pipetting up and down at least 20 times.
   Note: Avoid foaming of the solution!
3. Incubate the US IVD BTS solution for five (5) minutes at room temperature and repeat mixing by pipetting up and down at least 20 times.
   Note: Avoid foaming of the solution!
4. Centrifuge at 13,000 rpm for 2 minutes at room temperature. The US IVD BTS solution is now ready for use.

Storage of Reconstituted US IVD BTS Solution

If reconstituted US IVD BTS solution is not to be used immediately, store as described here:

1. Transfer 5 µL aliquots of the supernatant into screw-cap micro tubes (0.5 mL) and close tubes tightly. Date tube.
2. Store aliquots at -18°C/0°F or below. Frozen, dissolved US IVD BTS can be stored for up to 5 months.

EQUIPMENT AND SUPPLIES

1. Biological Safety Cabinet – Class II
2. Bench-Top Ultra-Fuge (capable of 13,000 to 15,000 rpm)
3. Vortex Mixer
4. Freezer (-18oC or lower)
5. Sterile Tooth Picks (pointed end)
6. MALDI Target Plate
7. Eppendorf Pipettes – Biopure or PCR Grade
   a. 0.5-20-µL
   b. 2-200-µL
   c. 50-1000-Ml
8. Eppendorf Sterile Disposable Pipette Tips – Biopure of PCR Grade
9. Eppendorf Plastic Tubes – 1.5-mL
10. Sarstedt Screw-Cap Micro Tubes
11. Paper Towels 12. Kimwipes
12. Disposable Inoculating Loop – 1-µL
13. Blank Target Plate Map

14. Digital Timer

INSTRUMENTATION

The standard MALDI Biotyper CA System contains the following components:

- microflex LT/SH mass spectrometer
- MALDI Biotyper CA System desktop computer running under Windows® 7
- MALDI Biotyper CA System software
- Uninterruptible Power Supply (UPS)
- Honeywell Hyperion 1300g

The Bruker microflex LT/SH mass spectrometer must be operated within a temperature range of +16°C/+61°F to +33°C/+91°F. The system performance is optimal within a temperature range of +18°C/+64°F to +25°C/+77°F.

QUALITY CONTROL

Internal Control

The control procedure requires that US IVD BTS is inoculated on the target at two or more positions (cross-joint positions are recommended). The MALDI Biotyper CA System microflex LT/SH mass spectrometer is automatically checked using US IVD BTS before each use. Only one of the prepared BTS control positions is automatically checked multiple times before the run proceeds. The following parameters are checked:

- Calibration peaks
- Spectrum baseline

The parameters are combined to provide an overall quality value from which the final control procedure outcome is determined. At the beginning of each test run, the control procedure confirms that the microflex LT/SH settings are appropriate. When the check is successful, the system automatically begins the measurement process. If the control procedure is not successful, the run is stopped and the message "QC on spot BTS(x) was not successful!" is displayed on the status bar; (x) represents a number from 0 to 4.

Documentation: The results of the Calibration Control are included with each stored run report.

Corrective Action for Quality Control Failure

If the control procedure fails, repeat the process using the second (unused) BTS control position on the same target (please clear the check box of the unsuccessful control position in the run).

If the control procedure using the second BTS control position fails, contact Bruker Technical Support.

After MALDI Biotyper CA System identification, please verify that BTS control positions are identified as *Escherichia coli* with log(score) ≥2.0. If one has “No Peaks” identification, please check whether the second BTS control position is identified as *Escherichia coli* with log(score) ≥2.0. If both BTS control positions have failed, the test run is not valid and must be repeated.

External Control

A. Organism Control – Run Once Daily per organism.

The following control strains will be run on each day of patient testing:

Control Strain	MALDI Identification	MALDI Score
S. aureus ATCC 43300	*Staphylococcus aureus*	≥2.0
C. albicans ATCC 10231	*Candida albicans*	≥2.0

Documentation: The results of the Daily Organism Control are entered in the LabPro Connect system and included in that stored run report.

Corrective Actions for Quality Control Failure:
If the Organism Control fails, repeat the process inoculating a second (unused) circle on the Target Plate. If the second control fails:

- Ensure that the correct c ontrol strain was used and was fresh and in pure culture
- Ensure optimal inoculum application
- Ensure that the matrix solution is within date and properly prepared
- If repeated failures occur, retrieve a new control strain from storage and place into use
- If the above steps fail to resolve the problem, notify Bruker Customer Support (1.877.410.2211) for assistance

B. Blank Control – Run once daily on each clean target plate.
Formic acid overlayed with Matrix solution will be applied on each clean Target Plate

Control	MALDI Identification	MALDI Score
Formic Acid/Matrix Solution	No Peaks Found	0.000

Documentation: The results of the Daily Organism Control are entered in the LabPro Connect system and included in that stored run report.

Corrective Actions for Quality Control Failure:
The Blank Control should generate no peaks. Any identification or score value may indicate that the Target Plate has not been properly cleaned. Re-clean the Target Plate and re-run the Blank Control. If this does not resolve the problem, notify Bruker Customer Support (1.877.410.2211)

C. Extraction Control – Run each day that a patient extraction specimen is run
Follow extraction procedure using the following organism:

Control Strain	MALDI Identification	MALDI Score
S. aureus ATCC 4300	*Staphylococcus aureus*	≥2.0

Documentation: The results of the Daily Organism Control are entered in the LabPro Connect system and included in that stored run report.

Corrective Actions for Quality Control Failure:
If the Extraction Control fails, repeat the process inoculating a second (unused) circle on the Target Plate. If the second control fails:

- Ensure that the extraction procedure was followed correctly including all reagents, solvents and consumables as specified by Bruker
- Ensure that the correct control strain was used and was fresh and in pure culture
- Ensure optimal inoculum application
- Ensure that the matrix solution is within date and properly prepared

- If repeated failures occur, retrieve a new control strain from storage and place into use
- If the above steps fail to resolve the problem, notify Bruker Customer Support (1.877.410.2211) for assistance

PROCEDURE

I. Sample Preparation

A. Direct Transfer (DT) Sample Preparation Procedure

1. Using a sterile colony transfer device, smear an isolated colony of bacteria or yeast as a thin film directly onto a sample position on a cleaned target.
2. On a blank Target Plate map – record the specimen accession number on the corresponding target position. Alternatively, affix a workcard barcode label to the corresponding target position.
3. Apply 1 µL of US IVD HCCA Matrix over inoculated spots. Use a new pipette tip to add matrix to each inoculated sample position. After samples have dried, US IVD HCCA Matrix must be added within 30 min or the target must be cleaned and the inoculation of samples must be performed again.
4. When specimen inoculation is complete and it is time to perform a run, select two BTS control positions on a US IVD 48 Spot Target to inoculate with 1 µL US IVD BTS solution and let dry at room temperature. Cross-joint positions are recommended for use as BTS control positions.
5. Overlay each of the BTS control positions with 1 µL US IVD HCCA Matrix. Use a new pipette tip to add matrix to each inoculated sample position. (US IVD HCCA Matrix must be applied within 30 min of the BTS drying or the target must be cleaned and the inoculation of samples must be performed again). Dry the inoculated target plate at room temperature.
6. The inoculated target plate is now ready for use. Note: An inoculated target plate must be processed within 24 hours of preparation or the target must be cleaned and the inoculation of samples and US IVD BTS must be performed again.

Note: Make sure that the screw-cap tube containing US IVD HCCA Matrix is tightly closed after use to minimize solvent evaporation.

The figure below illustrates suitable inoculum amounts of Gram-negative bacteria on target for successful MALDI Biotyper CA identification (Row A). In the case of Row B, the amount of test organism on the target was insufficient for MALDI Biotyper CA identification whereas in Row C, too much of the test organism was transferred.

Ideal (row A) and suboptimal (rows B and C) amounts of test organism on a target

B. Extended Direct Transfer (eDT) Sample Preparation Procedure

1. Using a sterile colony transfer device, smear an isolated colony of bacteria or yeast as a thin film directly onto a sample position on a cleaned target.
2. Overlay the sample spot with 1 µL 70% aqueous formic acid and allow to dry at room temperature.
3. Follow steps 2-6 above under section A to complete procedure.

C. Extraction (Ext) Sample Preparation Procedure

1. Transfer 300 µL of HPLC-grade water into an Eppendorf tube.
2. Using a sterile 1 µL inoculation loop, inoculate isolated colonies from the isolation plate originally used for the Direct Transfer sample preparation procedure or extended Direct Transfer sample preparation procedure into the water and mix thoroughly until the material is completely in suspension.
3. Add 900 µL of absolute ethanol and mix the suspension.
4. Spin down the microbial material in a bench-top centrifuge for two minutes at 13,000-15,000 rpm.
5. Remove the supernatant using a pipette.
6. Repeat step (4) and remove residual ethanol by pipetting (avoid contact with the microbial material).
7. Air-dry the pellet for at least five minutes at room temperature.
8. Add 25 µL 70% aqueous formic acid and pipet the solution up and down until the pellet is resuspended.
9. Add 25 µL of acetonitrile to the tube and mix by pipetting the solution up and down two or three times.
10. Centrifuge the tube for two minutes at 13,000-15,000 rpm.
11. Deposit 1 µL of the supernatant onto a sample position on a cleaned MALDI target plate.
12. Use a new pipette tip for each sample position.
13. Air-dry the target at room temperature.
14. Continue the procedure according to steps 2 -6 of the Direct Transfer sample preparation procedure.

Note: Extracted bacteria and yeast suspensions may be held at room temperature for up to 4 h prior to testing on the MALDI Biotyper CA System.

II. Loading and Ejecting Target

A. To load an inoculated target onto the MALDI Biotyper CA System:

1. Make sure that the target carrier is in the **OUT** position.
2. Open the load port lid and place the US IVD 48 Spot Target onto the target platform.

Note: If the lid does not open easily, the target carrier may be in the **IN** position. If this is the case, move the target carrier to the **OUT** position by pressing the MALDI target plate **IN/OUT** button once. Avoid dropping debris into the load port or onto the black O-ring. The presence of debris in or around the load port may result in poor vacuum.

3. Once the target has been loaded, wipe the O-ring/gasket with a gloved finger then close the lid.
4. Press the MALDI target plate **IN/OUT** button once.
5. The MALDI target plate loading procedure starts and the green **READY** and **ACCESS** LEDs go off.

6. The green **READY** LED will be lit again when the loading procedure had been successfully completed.

B. The MALDI target plate loading procedure typically takes two to three minutes. If the instrument is not ready after five minutes:
   1. Press the MALDI target plate **IN/OUT** button (see Figure 3-3) once to eject the target.
   2. When the green **ACCESS** LED is turned on, open and close the load port lid, ensuring no debris are on the O-ring/gasket.
   3. Press the MALDI target plate **IN/OUT** button (see Figure 3 3) once to reload the target.
   - If the problem persists, contact Bruker Technical Support.

Note: The green **READY** LED will be off and the yellow **WARM-UP** LED will be lit if the laser is in standby mode. The laser will enter this mode after 10 minutes with no acquisition. The green **READY** LED will be lit again when a new acquisition sequence is started.

C. To eject a target:
   1. Press the MALDI target plate **IN/OUT** button once. The MALDI target plate ejection procedure starts and the green **READY** and **ACCESS** LEDs are off.
   2. When the green **ACCESS** LED is lit again, open the load port lid, remove the target, and close the load port lid.

Note: The target carrier should only be moved to the OUT position when targets are being exchanged. Measured targets can remain in the instrument until the next target is ready to be processed. If no target is available, move the target carrier into the instrument (without a target in place) and move it out again only when the next target is ready to be loaded.
Note: When exchanging targets, do not leave the load port lid open for any longer than necessary. Leaving the load port lid open for extended periods does not damage the instrument, but will prolong target loading by as much as 30 minutes.

III. Operating the MALDI Biotyper CA System

A. Log on to the MALDI Biotyper CA System computer using the user name **ca-user**.
B. Start the MALDI Biotyper CA System Software by clicking the MALDI Biotyper CA System Software shortcut on the desktop. Alternatively, the MALDI Biotyper CA System Software can be started by selecting **Start > All Programs > Bruker Daltonics > MALDI Biotyper CA**.
C. After a few seconds the computer screen will indicate successful connection to the MALDI Biotyper CA System mass spectrometer and MALDI Biotyper CA System server in the status bar.
D. If the connection process fails, an error window will pop up offering further information. If the connection problem is not resolved an appropriate message will appear as a ToolTip below the status bar. If the connection process continues to fail after repeated startup attempts, contact Bruker Daltonics Inc. Customer Service.

IV. Performing a Test Run

A. Scan the unique ten-digit target plate ID number into the **Target ID** box and press the Enter key. Once this step is performed, the run cannot be edited further.
B. After the target ID has been entered, the run created in the LabPro Connect MBT Monitor (Refer to LabPro MBT Software procedure) will populate on the screen.

C. Ensure the target plate that was loaded into the MALDI Biotyper is the same target plate that is assigned on the screen.
D. After the vacuum has been made (Source High Actual< Source High Setpoint), select Start Acquisition to process the run.
E. During start-up, auto-calibration is performed and US IVD BTS is processed to confirm that the BTS control procedure meets all defined specifications. The status of the control procedure is displayed in the status bar at the bottom of the MALDI Biotyper CA System window.
F. After successful measurement of the BTS control position, sample positions are processed. The status bar displays the number of test organisms and identified sample positions and the time remaining for the measurement process and the identification process.
G. <u>If the BTS control procedure fails:</u>
    1. Observe the status bar to see whether the second BTS control position is now being used (the MALDI Biotyper CA System selects from the defined BTS control positions according to internal rules).
    2. If the second BTS control procedure is also performed on BTS control position that previously failed, you can stop the run and restart it to have the MALDI Biotyper CA System select the unused BTS control position,
    3. If the control procedure using the second BTS control position fails, contact Bruker Daltonics Inc. Customer Service.
H. MALDI Biotyper CA System identification consists of two steps, which are performed on each test and BTS control position:
    1. A mass spectrum is acquired from the test or BTS control position (measurement step).
    2. The resulting mass spectrum is processed and the resulting peak pattern is matched against reference patterns in the MALDI Biotyper CA Reference Library (identification step).
I. The identification step is started immediately after the associated measurement step has been completed and a mass spectrum is available.
J. When the identification run is started, the sample positions to be measured appear as white circles in the target display
K. During the run, the appearance of the sample positions and BTS control positions in the target display reflects the success of the measurement and identification process of each test organism.
L. If spectrum measurement is successfully completed, the left half of the sample position is colored green. If measurement fails, the left half of the sample position is colored orange.
M. The coloring of the right half of the sample position indicates the score value of the identification (green, yellow or red). The legend display explains the color-coding used to indicate the status of sample positions in the target display.
    1. Point to the information button in the top-right corner of the target display to show the color-coding legend
N. When the identification procedure is completed, the organism identification result will appear in the result table. If a non-clinically validated reference pattern (MSP) can provide an organism identification with confidence higher log(score), the name of the non-clinically validated reference pattern (MSP) and the log(score) of the match will

appear at the beginning of the description field in square brackets [(Listeria monocytogenes (2.26)].

Note: Non-clinically validated organism results are created from reference patterns which have not been clinically validated. Identification of non-clinically validated organisms must be performed with an alternate laboratory method. Results for non-clinically validated organisms cannot be transmitted from the MALDI Biotyper CA System to the laboratory information system.

The result table of the MALDI Biotyper CA System window gives a real-time overview of the identification results for the active test run. This table contains only the best matching reference pattern for each test organism and is a summary of the complete Run Results Report.

Tip: A PDF result report can be generated at any time by clicking the **View Results Report** button or **View > Results** on the menu bar.

When measurement and identification of all sample positions have been completed, the status bar displays the status message Finished successfully.

Note: When real-time identification is started for the first time after a reboot of the computer, it may take a while for the identification results to be generated. This is due to the reference patterns being loaded from the database into a cache in the computer memory.

V. Target Cleaning Reagent Preparation

Please prepare the solutions required for cleaning targets as follows:

A. Preparation of 70% aqueous ethanol
    1. To prepare 100 mL solution, measure 30 mL HPLC-grade water with a graduated cylinder.
    2. Transfer the water into a beaker.
    3. Measure 70 mL absolute ethanol and mix with the water in a beaker.
    4. Generate a homogeneous mixture by transferring the mixture from the beaker into the graduated cylinder and back again.
    5. Repeat step 4 five times.
    6. The solution is ready for use.

B. Preparation of 80% aqueous trifluoroacetic acid
    1. Transfer 200 µL of HPLC-grade water into a 1.5 mL Eppendorf plastic tube.
    2. Carefully add 800 µL trifluoroacetic acid.
    3. Close the tube tightly.
    4. Mix by inverting 5 times.
    5. The solution is ready for use.

C. Target cleaning procedure
    1. Transfer the target(s) into a suitable container or onto sufficient absorbent material and cover the surface with 70% aqueous ethanol.
    2. Incubate for five minutes at room temperature.
    3. Remove the target and rinse it thoroughly under running tap water.
    4. Using a Kimwipe, clean the target thoroughly with 70% aqueous ethanol.
    5. Rinse the target with tap water and wipe it with a Kimwipe.
    6. Cover the target with a layer of 80% aqueous trifluoroacetic acid (prepared as

described above) by adding 100 µL with a pipette and thoroughly wipe all US IVD 48 Spot Target plate positions with a Kimwipe.

7. Rinse the target with HPLC-grade water and wipe it dry with a Kimwipe.
8. Let the target dry completely for at least 15 minutes at room temperature.
9. Store the clean target in the container provided.

D. Storing Cleaned Targets

1. Cleaned targets can be stored before use in a dry place at room temperature in the container provided. Avoid exposing cleaned targets to potential contamination (for example, dust) or corrosive atmospheres.

Note: Do not place any adhesive labels on the target. Do not drop or scratch the target.

MAINTENANCE

1. *Daily*: Keep the load port area free from dust and debris. Use a soft lint-free cloth to wipe the area. DO NOT USE Kim- Wipes, Kleenex or any other lint-producing item.
2. *Weekly*: On Sunday, perform a source cleaning. (Flex Control►Status Tab►Details►Source Cleaning) and reboot the PC. Maintenance Interval Document on the appropriate log sheet.

RESULTS

Calculation

The spectrum of the unknown test organism, acquired through the MALDI Biotyper CA System Software, is electronically transformed into a peak list. Using a biostatistical algorithm, this peak list is compared to reference peak lists of organisms in the reference library (database) and a log(score) value between 0.00 and 3.00 is calculated. The higher the log(score) value, the higher the degree of similarity to a given organism in the reference library. A log(score) value ≥2.00 can be considered as a high-confidence identification of the test organism. A log(score) value between 1.70 and 1.99 can be considered as a low confidence identification of the test organism.

Interpretation

The log(score) value ranges defined in the MALDI Biotyper CA System reflect the probability of organism identification. Results should be reviewed by a trained microbiologist and final organism identification should be based on all relevant information available. This information includes, but is not limited to, Gram staining, colony morphology, growth characteristics, sample matrix, or other factors that might impact organism identification.

Range	Color	Interpretation	Additional Action
2.00 – 3.00	**Green**	High Confidence Identification	None
1.70-1.99	**Yellow**	Low Confidence Identification	If a direct transfer was used, repeat using both a direct transfer and extraction preparation
			If an extraction preparation was used, report as a "Low Confidence Identification" or identify by an alternate method
< 1.70	**Red**	No Organism Identification Possible	If a direct transfer was used, repeat using both a direct transfer and extraction preparation.
			If an extraction preparation was used, identify by an alternate method.

Reporting

Organisms can only be reported of a log(score) value is 2.00 or greater. Only organisms that are

identified on the CA database can be reported.

TROUBLESHOOTING

Scenario	Potential Cause	Recommended Action
No Peaks Generated	Incomplete sample preparation (no sample and/or matrix added)	Repeat testing after reviewing the procedure and ensuring that all required steps are performed.
	Incorrect sample preparation (excessive or insufficient amount of inoculum)	Repeat testing taking care to check the inoculum size – spot in duplicate with varying amounts.
	Problematic consumables (polymers leached from plastics, expired reagents, dirty Target Plates)	Use only the consumables specified in this procedure. Do not use reagents beyond the stated expiration date. Clean Target Plates thoroughly.
	Test organism	Subculture the test organism and repeat testing (must be performed within 12 hours).
Log Score Values <2.0	Incorrect sample preparation (excessive or insufficient amount of inoculum)	Repeat testing taking care to check the inoculum size – spot in duplicate with varying amounts.
	Problematic consumables (polymers leached from plastics, expired reagents, dirty Target Plates)	Use only the consumables specified in this procedure. Do not use reagents beyond the stated expiration date. Clean Target Plates thoroughly.
	Test organism	Subculture the test organism and repeat testing (must be performed within 12 hours).
	Test organism not included in the reference library	The test organism may not be included in the reference library and therefore cannot be identified.
Automatic Measurement Interrupted	Power outage	Call Bruker Customer Service (1.877.410.2211) for guidance in starting up the MALDI Biotyper and resuming the interrupted identification run.
Missing Identification Results For Measured Samples	The identification result message generated by the server is not displayed in the MALDI Biotyper Clinical Application window	No data is lost. To recover the missing identification results, reload the test run or use the **View > Results** function to generate a report. The PDF report will contain the positions missing in the target display.
A New Run Cannot Be Created	The internal logic of the software allows only to have one run of status prepared for a certain target plate. Therefore, the previously defined but still unmeasured run is present for measuring.	Either measure the old run or if this is not possible hide the old run to allow the creation of a new run for this target plate. Hiding a run of prepared status is done in the **Open Run** pop up table by clicking the **Hide Run** button right to the **Report** button. If the column with the **Hide Run** button is not visible it can be activated by right-clicking the table header and selecting the appropriate check box.

PROCEDURAL NOTES

- Inoculating an appropriate amount of the test organism onto the target is important. Excessive or insufficient amounts of inoculum may impact organism identification.
- An inoculated target must be processed within 24 hours of preparation or the target must be cleaned and the inoculation of samples and US IVD BTS must be performed again.
- Organisms for testing must be isolated on one of the recommended media. Organisms for testing must be subcultured as necessary to ensure purity. Use only a single isolated colony when performing identification on the MALDI Biotyper CA System.
- Chemicals used in this procedure may be highly flammable, corrosive and harmful. Read the relevant Material Safety Data Sheets provided by the manufacturer. Wear appropriate personal protective equipment when handling these chemicals. Always work inside a fume hood if recommended by the reagent supplier.
- Follow all instructions carefully. Improper sample preparation or cleaning of the Target Plate may lead to erroneous results.
- Testing must be performed using an isolated colony. Testing mixed cultures could lead to erroneous results.
- A test organism identification with a log (score) value of less than 2.00 should not be considered a reliable identification. Additional testing, including sample extraction is required.
- A result of "No Peaks Found" indicates that no spectrum is available. Testing should be repeated. Make sure that all instructions are carefully followed.
- Certain test organisms may be identified only to the genus level. See *MALDI Biotyper CA System Package Insert Reference Library* for reference library information.
- The instrument cover should never be removed except by certified service personnel. Removing the cover by untrained personnel may result in eye damage from laser exposure.

LIMITATIONS:

- Testing cannot be performed directly on patient specimens.
- Testing cannot be performed on mixed bacterial cultures.
- Testing cannot be performed directly from a liquid broth.
- Organisms will not be identified if there is no reference pattern in the reference library for the test organism.
- All identifications of non-lactose fermenting *Escherichia coli* will need to be tested by indole reaction. If indole is positive, isolates should be run on the Rapid Neg ID and identified from MicroScan. If the indole is negative, the isolate can be identified as *Shigella species* and sent for confirmation to the state. Additional tests for low discrimination results are necessary for the completion of the organism identification.

REFERENCES

MALDI Biotyper CA System User Manual. Bruker Daltonics Inc., Billerica, MA. September 2017.

MALDI Biotyper CA System Package Insert Reference Library 3.2. Bruker Daltonics Inc., Billerica, MA. September 2017

UnityPoint Proctor Laboratory is a CAP accredited facility. As of July 1, 2011, the responsibility of new and/or substantially revised policies and procedures will be restricted the Laboratory Director whose name appears on the CLIA certificate, and the signature appears below. The biennial review will be completed by the appointed Section Medical Director.

POLICY CREATION :		
Author:	***Audrey Davis***	***March 28, 2018***
Medical Director:	***Elizabeth A. Bauer-Marsh*** Elizabeth A. Bauer-Marsh MD	***March 28, 2018***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
March 27, 2018	Elizabeth A. Bauer-Marsh, M.D.	Elizabeth A. Bauer-Marsh MD
SECTION MEDICAL DIRECTOR		
March 27, 2018	Lori Racsa, DO	L Racsa D.O.

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Initial Release	A. Davis	3/27/18

REVIEWED BY

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
Marsha Bishoff	3/30/18	Audrey Davis	3/27/18	L Racsa D.O.	3/27/18