PROCEDURE: **VITEK MS**

I. PRINCIPLE

The VITEK MS is a matrix-assisted laser desorption ionization-time of flight mass spectrometer for rapid identification of microorganisms from culture. The sample is uniformly mixed with matrix. The sample crystalizes in the matrix and the matrix protects the sample from being completely destroyed by the 337nm ultraviolet nitrogen laser. The matrix also aids in desorption of the specimen when the laser is applied. It transfers positive charges to the sample after being in contact with the laser beam.

After contact with the laser, the sample is vaporized and because the sample is now positively charged, the sample, along with a small portion of matrix, is guided into the vacuum tube by the negatively charged field. The velocity of the particles in this vacuum tube depends on the mass/charge ration of the particles. The time that it takes for the particles to travel a known distance, referred to as “time of flight”, of the particles is measured. Heavier particles travel slower. This data is collected by the ion detector.

A spectrum is created in the computer of the masses that are detected. Each organism has a unique spectrum. The obtained spectrum is compared to the VITEK MS knowledge base and organism identification is applied.

II. SPECIMEN COLLECTION AND PROCESSING

A. Pure colonies from a primary or restreak plate on which the growth is 24-72 hours old
B. Only growth on validated media can be used
    1. 5% Sheep Blood Agar
    2. Chocolate Agar
    3. MacConkey Agar
    4. CNA agar
    5. Sabouraud Dextrose Agar
    6. Brucella Agar

III. EQUIPMENT AND MATERIALS

A. VITEK MS-DS target slides –Room Temperature (15-25°C)
B. VITEK MS-CHCA (matrix) – Refrigerated (2-8°C)**,** stabile for 7 days once opened
C. VITEK FA (formic acid) REAGENT – Refrigerated (2-8°C), stabile for 14 days once opened
D. 1µl calibrated green plastic loops
E. VITEK PICKME pen and nibs
F. Precision pipette to deliver 1.0µl (for matrix)
G. Precision pipette to deliver 0.5µl (for formic acid)
H. Non-sterile colorless pipette tips without filter

IV. QUALITY CONTROL

A. *K. aerogenes* ATCC 13048
B. *Candida glabrata* MYA-2950
C. *E. coli* ATCC 8739 (for calibration spot and fine tuning)
D. Refer to VITEK MS QC PROCEDURE for information regarding daily QC, fine tuning, and reagent QC

V. SAMPLE SLIDE PREPARATION PROCEDURE

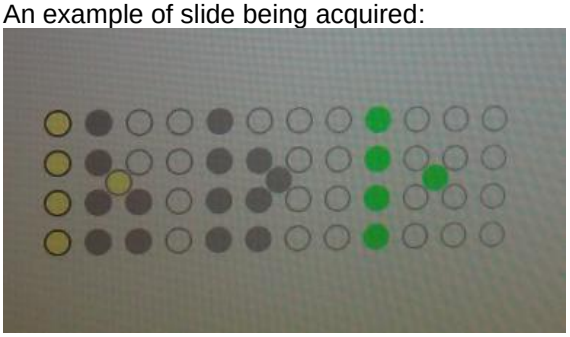
A. Use a 1µl green plastic loop or Vitek PICKME pen and nib to apply *E. coli* ATCC 8739 to the small control/calibration spot in the center of each acquisition group being used.

B. Pipette 1.0µl of VITEK MS-CHCA matrix immediately onto the spot and allow to air dry.
C. Use a 1µl green plastic loop or Vitek PICKME pen and nib to pick up part of the colony.
D. Inoculate the selected colony on the two designated isolate spots. Avoid excessive inoculation.
E. ***IF YOU ARE PROCESSING A FUNGAL ISOLATE***: Immediately, **pipette 0.5µl of VITEK MS-FA reagent** to the center of each yeast spot and allow that to **dry completely**. Be sure to use a new pipette tip for each spot.
F. Pipette 1.0µl of VITEK MS-CHCA matrix on to the center of each prepared spot. Use a new pipette tip for each spot.
G. Allow matrix/organism suspension to dry completely on all spots. When dry, the spots will have visible crystal formation (a yellow film).
H. Repeat steps C-G for all additional isolates that need to be run.
I. Once all spots are dry and the slide is programmed, it can be loaded onto the instrument.

VI. SAMPLE SLIDE FLEXPREP PROCEDURE

A. Open and log into FlexPrep.
B. Click the Layout icon until Vitek MS is displayed.
C. Scan in the Vitek MS-DS target slide.
D. Keep the default IVD preparation mode method.
E. Enter the matrix and formic acid lot numbers used.
F. Select the appropriate bench and set up operator.
G. Scan the specimen accession number.
H. Verify the correct isolate number.
I. Verify that the appropriate bacteria/fungi setting is being used for that isolate.
J. Click Validate.
K. Repeat steps G-J until all the prepared spots are linked to a location on the slide.
L. Check that all spots used are validated and then click the Close Slide button in the Flex Prep software.
M. At the Acquisition Station, log into the Acquisition Station software.
N. Click the Open button on the Acquisition screen.
O. Once the door opens, remove the adapter from the VITEK MS and remove any old slides. Discard any slide on which all three acquisition groups have been used.
P. Load the target slides to be tested in the adapter. The top of the adapter has angled corners and is the closest to slide position 1 with barcodes facing out to the left.
Q. Scan the target slide barcodes starting with the slide in position 1.
R. Load the adapter into the VITEK MS with the angled corners into the machine and slide the adapter until it will go no further. The edge of the adapter should be flush to the machine when properly loaded.
S. Click the Start button to run the slides.
T. Once the target slides have completed the run and QC has passed, the isolates can be reviewed in the MYLA software.

An example of slide being acquired:



Guide for spots at acquisition station:

SPOT	COLOR	DESCRIPTION
	Grey	No sample programmed
	Dark grey	Previously used aquisition
	Dark blue	Waiting for aquisition
	Light blue	Spectrum is being acquired
	Green with green border	Spectrum acquired; quality checks passed; sent to Myla.
	Green with red border	Spectrum acquired; quality checks passed; not sent to Myla
	Red with green border	Spectrum acquired; quality checks failed; sent to Myla
	Red with red border	Spectrum acquired; quality checks failed; not sent to Myla
	Light yellow	Spot selected for re-aquisition

IX. REVIEWING/INTERPRETING RESULTS

A. Log-in to VITEK MS Portal home page.
B. Click on Vitek MS Software icon.
C. Click the Results to Review icon to access the VITEK MS Review screen.
D. **Only identifications that match the colony morphology and preliminary tests should be reviewed and released. ALL spot tests and any additional testing must be recorded in the worksheet.**

i. High confidence results:

 - Good identification.

1. OK to validate as long as result is consistent with colony morphology.
2. Additional testing may be required for isolates that have been flagged as highly pathogenic or critical pathogens.

ii. Medium confidence results:

 - Low discrimination.

1. Slash-line/low discrimination results have occurred between the two spots for the isolate. Identification may be made by an alternate method or a spot test/additional testing can help to identify the organism.
2. <u>Discrepancy due to a good ID on one spot and a bad ID on the other:</u>
    a. The good identification may be accepted if the results correlate with the colony morphology and spot tests.
3. <u>Discrepancy due to contradicting IDs (ex. GNR and GPR):</u>
    a. Neither of the identifications should be reviewed or accepted. Vitek MS spots should be repeated, or identification should be made by an alternate method. If the repeated VITEK MS is still discrepant, bring the culture up on Rounds.

iii. No results:

 - No identification.

1. Reject these results and perform the identification by an alternate method.

iv. Non-FDA Approved results:

1. This laboratory has developed a database of identifications that are acceptable for use. A director approved current list can be found at the benches. These identifications MUST be used in conjunction with the isolate comment:
   "This organism has been identified using a FDA cleared MALDI-TOF MS using a laboratory-developed database determined by the Clinical Microbiology Laboratory at Lifespan Academic Medical Center."
2. **If organism is not in laboratory database, bring up on rounds if non-FDA approved identification needs to be used.**
   a. The identification may be accepted under the review of the Medical Director/Associate Medical Director/designee if it correlates with culture growth or clinical picture. The comment below should be used:
   "Identification has been performed by a method that has not been cleared by the FDA. The result is consistent with culture growth and organism characteristics."

E. The interpretation of VITEK MS results and the use of the instrumentation require a competently trained technologist. Judicious interpretation of results based on technologists' experience, species information and other pertinent tests should be utilized when reporting the identification of tested organisms. Any additional information that is known to the technologist (gram stain reaction and morphology; colony morphology; atmospheric growth requirements; spot test results) should be considered before the results of the VITEK MS are accepted.

X. PROCEDURE NOTES

A. To avoid sample drying, no more than two spots should be prepared before adding matrix or formic acid.
B. The samples on the target slides should be dry before placing into the instrument. Failure to dry the slide will cause the instrument to take an extended amount of time to pump down to the appropriate working pressure.
C. Gloves should be worn at all times when handling the target slides.
D. Ambient laboratory temperature above 26°C may affect test performance. The calibration may drift causing erroneous or undesirable results. The Inlet temperature is recorded daily to monitor this temperature.
E. The quality of results produced is dependent on a good sample preparation step.
F. Fine tuning is required to avoid the calibration from drifting. The schedule for fine tuning can be found on the QC calendar whiteboard/MALDI PM binder for scheduled dates. A field service engineer will perform this scheduled fine tuning. This tuning should take approximately 4 hours.
G. If the instrument starts to take an exceeding long amount of time to attain the correct pressure or acquire the 100 peaks needed to make identification, a fine tuning of the instrument may be needed.
   i. Notify the manager or a Technical Specialist. Call BioMerieux customer service (1-800-682-2666) for guidance and fine-tuning protocol. You cannot fine tune without calling BioMerieux. They will need to gain remote access and they will give instructions.
   ii. They may need a calibration slide prepared. Refer to Section VII – Calibration Slide Procedure.
H. When preparing your slide, picking up agar along with the colony may lead to poor identification results.

I. If the quality control spot fails upon the second check during the assay, a fine tuning may be required.
J. Spots may be re-fired no more than twice.

XI. LIMITATIONS

A. Only validated microbial Isolates can be identified. Refer to manufacturer list for specific organism.
B. The system is not validated for use with direct patient samples or other sources containing mixed flora.
C. Additional testing is required for low discrimination (slash-line/low confidence) or non-clinically validated organisms.
D. *Shigella* species and *E. coli* 0157 are identified as "*Escherichia coli*"– testing is required to differentiate. Non-lactose fermenting colonies on MacConkey agar suggestive of E. coli should not be identified using the Vitek MS.
E. Confirmation tests are recommended for *Neisseria gonorrhoeae*. Bring up on Rounds. The VITEK NH card can identify this organism.
F. *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* should be considered as *Aeromonas* species group and further testing needs to be performed VITEK GN card can identify this organism.
G. *Achromobacter dentrificans* and *Achromobacter xylosoxidans* should be considered a slash-line. Identification needs to be confirmed before reviewing results. The VITEK GN card may help identify these organisms.
H. *Enterobacter cloacae* and *Enterobacter asburiae* should be considered slash-line identification. It is acceptable to identify these isolates as *Enterobacter cloacae complex*. Further identification may be performed by VITEK GN card.
I. *Proteus penneri* and *Proteus vulgaris* results should be considered a slashline. A spot indole needs to be performed to confirm identification before review.
J. Organisms identified as *Citrobacter freundii, Citrobacter braakii* or *Citrobacter youngae* should be considered as *Citrobacter freundii complex*

XII. REFERENCES

VITEK MS User Manual
www.biomerieux-industry.com

VITEK ® MS V3.1 "The Basics…"
RPN 056470 – Rev. 01.A

XIII. REVISIONS

A. 6/14/24 Removed media that is no longer in use, moved QC information to Vitek MS QC Procedure, updated sample slide prep procedure, added sample slide FlexPrep procedure, general reformatting