PROCEDURE: VITEK MS QC

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 ("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. 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

III. QUALITY CONTROL ORGANISMS

A. *K. aerogenes* ATCC 13048
B. *Candida glabrata* MYA-2950
C. *E. coli* ATCC 8739 (for calibration spot and fine tuning)

IV. QC SLIDE PREPARATION PROCEDURE

A. QC preparation must be done on a new, empty slide.
B. 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.
C. Pipette 1.0µl of VITEK MS-CHCA matrix immediately onto the spot and allow to air dry.
D. Apply *K. aerogenes* ATCC 13048 to spot A1 and add 1.0µl of VITEK MS-CHCA matrix.
E. Apply *Candida glabrata* MYA-2950 to spot A2 and add 0.5µl of VITEK MS-FA reagent. After this spot is dry add 1.0µl of VITEK MS-CHCA matrix.
F. Apply 0.5µl of VITEK MS-FA reagent to spot A3. After this spot is dry add 1.0µl of VITEK MS-CHCA matrix.
G. Apply 1.0µl of VITEK MS-CHCA matrix to spot A4.
H. Once all spots are dry and the slide is programmed, it can be loaded onto the instrument.

V. QC SLIDE FLEXPREP PROCEDURE

A. Open and log into FlexPrep.
B. Scan the slide ID.
C. Enter the matrix and formic acid lot numbers used.
D. Choose QC as the bench.
E. Click the QC button.
F. Open the drop-down menu and select Daily.
G. Select the setup operator.
H. Click validate.
I. Load slide onto instrument.

VI. REVIEWING/INTERPRETING QC RESULTS

A. Open and log into VITEK MS software.
B. Click results then To Review.
C. Search by bench and choose QC.
D. Ensure the *K. aerogenes* ATCC 13048 and *Candida glabrata* MYA-2950 spots were properly identified and validate.
E. Ensure the VITEK MS-FA and VITEK MS-CHCA spots did not ID and reject.
F. Any QC that is not acceptable must be repeated. Notify the specialist if undesirable QC results persist as an unscheduled calibration may be necessary.

VII. CALIBRATION SLIDE PREPARATION PROCEDURE

A. Fine tuning is performed once a month, refer to the QC calendar whiteboard/MALDI PM binder for scheduled dates.
B. Obtain a new target slide.
C. Open a new VITEK MS-CHCA matrix container.
D. Place 1µl of VITEK MS-CHCA matrix to spot A1 **only.**
E. Place the working QC strain of *E. coli* ATCC 8739 followed by VITEK MS-CHCA matrix on all of the remaining spots of the target slide.

VIII. CALIBRATION SLIDE FLEXPREP PROCEDURE

A. Open and log into FlexPrep.
B. Scan the slide ID.
C. Enter the matrix lot number used.
D. Choose QC as the bench.
E. Select the setup operator.
F. Program only the first acquisition in the Vitek FlexPrep using premade barcodes for fine tuning.
G. Load the fine tuning slide in the first spot on the tray and a blank slide in the last spot..
H. **CLOSE BUT DO NOT START THE MALDI.**

IX. SUBBING QC ORGANISMS

A. *K. aerogenes* ATCC 13048, *Candida glabrata* MYA-2950, and *E. coli* ATCC 8739 are subbed from fresh loops biweekly for mother plates**.** Organism loops are stored in the walk in fridge.
B. Inoculate one BAP with *K. aerogenes* ATCC 13048 and date for 2 weeks.
C. Inoculate one SAB with *Candida glabrata* MYA-2950 and date for 2 weeks.
D. Inoculate two BAPs with *E. coli* ATCC 8739 and date for 2 weeks (one week for each plate).
E. Broth method for inoculation:
    1. Transfer each loop to a BHI and put in incubator for just long enough for the film to completely dissolve out of the loop.
    2. Shake tube gently to suspend the organism.

3. Inoculate plates using BHI and streak for isolation.

F. Direct from loop for inoculation:
   1. Stab loop into first quadrant of plate and leave for 10-15 seconds.
   2. Streak plate for isolation.

G. All mother plates are stored at 2-8°C for 2 weeks.
H. *E. coli* ATCC 8739 is subbed fresh from mother plate every day.

X. REAGENT QUALITY CONTROL

A. New lots of VITEK MS-CHCA matrix and VITEK MS-FA are recorded upon receipt and QC'ed during daily QC.
B. Once the results are confirmed the lots can be used.

I. 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.
   1. 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.
   2. 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.

X. REFERENCES

A. VITEK MS User Manual www.biomerieux-industry.com VITEK ® MS V3.1 "The Basics…" RPN 056470 – Rev. 01.A
B. Culti-Loops QC Organisms. Lenexa, KS: Remel, Inc. 2022.