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Prepared by QA Committee		
Issued by: Laboratory Manager	Revision Date: 7/25/2018	
Approved by Laboratory Director: Microbiologist-in-Chief	Annual Review Date: 5/1/2019	

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Procedure of MALDI

VITEK MS Slide spot preparation

Before beginning to apply spots on a Vitek slide ensure slide is clean and reagents (matrix and formic acid) are at room temperature.

1. Label the [Vitek MS Worksheet](#) or [Vitek MS Worksheet with Controls](#) with the date and slide number
2. Apply a fresh isolate (24 hrs) of E. coli ATCC 8739 control in the central circle of the slide using 1ul of green loop for each acquisition group used.
3. Add 1ul of Matrix. Do not invert the matrix vial. Do not touch the crystals on the bottom of the vial with pipette tip
4. Ensure plates to be tested match the accompanying LIS label
5. Place one specimen barcode label on the work sheet
6. Apply a thin even layer of organism in each circle of the slide by using 1ul of green loop or wooden toothpick
7. Add 1ul of Matrix. Do not invert the matrix vial. Do not touch the crystals on the bottom of the vial with pipette tip
8. For Yeast, add 0.5ul of formic acid first, wait until it crystallizes, then add 1ul matrix,

VITEK MS Prep Station (touch screen option)



1. Double click Vitek MS Prep Station icon
2. Enter User name: labtech
3. Enter Password: Labtech# e.g. "Labtech1"
 - o The password # changes, check bench for posting of current password #)
4. Click green check mark or press enter
5. Enter slide id# by scanning the slide barcode

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6. Scan the specimen barcode on the plate in the Lab id# field
 - Bacteria is the default option
 - For yeast spots, choose Fungi option
7. Press F4 to save or touch Validate green check mark
8. Click Send Slide arrow after all the specimens have been applied
9. Choose Yes in Select an Option window
10. Ensure no slides pending review in the instrument before loading (check clipboard).

VITEK MS Acquisition Station



1. Put on a new pair of gloves
2. DOUBLE CLICK Vitek MS Acquisition window
3. Enter User Name: labtech
4. Enter Password: Labtech# e.g. "Labtech1"
 - The password # changes, check bench for posting of current password #)
5. Click Validation Check Mark or press Enter
6. Click Open button on the upper right side of the computer screen
7. Pull out slide adaptor once the Vitek MS door is open
8. Remove the old slide from the adaptor
 - If slide removed has unused acquisition group(s) - place on partial sorting shelf
 - If all acquisitions on slide removed have been used (all spots finished) – discard slide
9. Put the new slides to be tested slide in the position 1,2,3,4
10. Scan the slide id#
11. Load the slide into Vitek MS machine (barcode to the left side) with one hand push down the metal bar and the other hand slide in the dented side into the machine
12. Press Start button to start the acquisition (if unable to select Start option, you may not have sent your slide information from the prep station, you can send it now)

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13. Place MS worksheet on clipboard labeled as “Pending Review”

MYLA (Result Review)



1. Click Dashboard-Window Internet Explorer (MYLA) window
2. Enter User Name: labtech
3. Enter Password: Labtech3 (the password may change, check bench for posting of current password)
4. Click Login button or press Enter
5. Dashboard window will appear
6. Click Vitek MS Review
7. VITEK MS Review will appear
8. Under bench name, click arrow and choose either Prepstation 1 or Prepstation 2 or whichever prep station you used to enter the barcodes on the slide
9. Under Setup Date, select today’s date from and today’s date to
10. Press Search button
11. Review results according to confidence level
12. In Confidence Level field, click the arrow down and select Red dot (low) as 1st step
13. Circle the lab# for further re-fire
14. Click the check mark on the first left row, choose trash can icon, press OK
15. Select yellow triangle (medium) as 2nd step
16. View the result by clicking the Accessing ID#. Print screen showing the possible organisms. Refer to the MS manual for commonly occurring low discrimination results. Where possible, choose the designated organism. Choose one id only by clicking the round circle on the first left row. Click the appropriate box. Return to MS review. Do this for each organism with a

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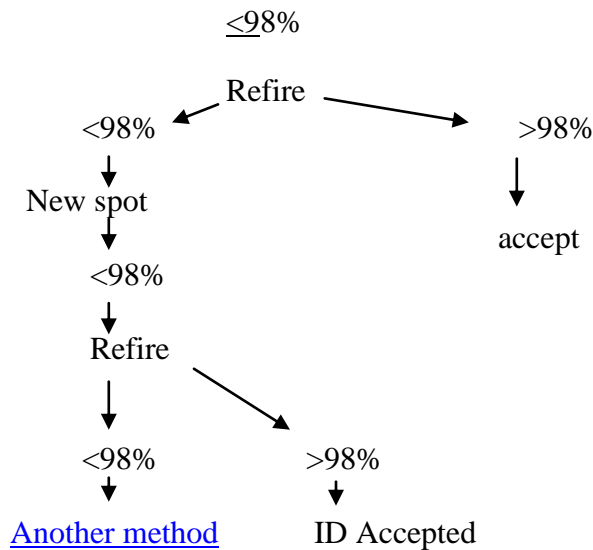
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yellow triangle. For those with vastly different organism ID options and/ or implausible ID's, return to MS review and send to trash. For the ones sent to trash, circle the lab# for further re-fire



17. Meanwhile Select All as 3rd step
18. Check the confidence % for all the isolates with green squares. Any that are <98% should be re-fired. Circle the lab# for re-fire
19. Refiring: Take MS worksheet to the Acquisition station computer. Log on. Using the mouse, select all of the circles which have been circled and click Start. Meanwhile print all the results by using printer icon- move this statement to end of #18 or 18A
20. If =/> 98%, continue to next step.
21. Select all isolates \geq 98% or which have been completely reviewed. Click the green check mark (Review Selected Results icon) to complete the review.
22. After re-firing review the results as above. Those that are still red circles, examine the graph to access the quality of the peaks. If the peaks are good, choose other ID method. If the peaks are not good, an ID may not be acquired due to be a poor prep. It may be worthwhile doing another preparation.
23. For those that are yellow triangles, follow through as above
24. Click the Vitek MS Review to go back previous page
25. All the results should be reviewed and cleared from the review list
26. Put the Matrix and formic acid into the fridge at the end of the day. Close the system
27. Note: You can return to the dashboard at any time by clicking on the ‘house-shaped’ icon
28. If looking for a result by specimen, click on the binoculars in MYLA, then choose tab “Specimen” and enter LIS number (can scan barcode) and click on Search. Drag the blue slide down to view.

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Tips:

- Mucoïd colonies: use sterile swab to take off the mucoïd layer and then use the loop to pick the colonies.
- Extract all the Yeast with formic acid.
- Make confluent monolayer on the spot
- Try using loop first, if difficult to pick the colonies, and then use stick.

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MALDI Organism Identification Acceptance

1. Common isolates with $\geq 98\%$ report as id by MYLA assuming that the colonial morphology or gram appearance matches the identification
2. Uncommon isolates with MYLA confidence level of $\geq 98\%$ - report as presumptive with additional in-house testing and confirmation by PHL as necessary assuming that the colonial morphology or gram appearance matches the identification.
See file “Vitek MS Uncommon bacteria to confirm at PHOL” located in the T:drive T:\microbiology\Vitek MS\
3. For identifications that appear as “low discrimination” by MYLA that has a list of a few isolates of the same genera, report as the complex or species based on the full list of Vitek MS organism identifications, Myla organism names and our LIS translated names: [VITEK-MS-V2-speciesList Created-Translated list](#)
4. Always report the following as presumptive with additional testing for confirmation by PHOL assuming that the colonial morphology or gram appearance matches the identification
 - *Cardiobacterium hominis*
 - *Haemophilus haemolyticus*
 - *Helicobacter pylori*
 - *Peptoniphilus asaccharolyticus*
 - *Mycoplasma* species (*Mycoplasma bovis*, *Mycoplasma hominis*, *Mycoplasma hyorhithinis*)
 - *Yersinia pestis*
 - *Bacillus anthracis*
5. Never to accept the following organism identifications from MYLA – report Gram stain morphology only
 - *Listeria innocua*
 - *Listeria grayii*
6. For any GAS, GCS, GGS identified by MALDI, confirmation by Agglutination is needed before reporting.

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7. For NLF *E coli* identified by MALDI, confirmation by] is needed before reporting.
- If [Wellcolex Shigella Latex](#) is negative, report *E.coli*
 - If [Wellcolex Shigella Latex](#) is positive test with Remel *Shigella* serology and follow reporting algorithm in the table below:

Wellcolex	Remel	Further Workup
POS	POS	<ul style="list-style-type: none"> • Release <i>Shigella species</i> with isolate comment \shcn “presumptive isolated. NOTE: Occasionally, some E. coli may identify as presumptive Shigella species using our current in-lab methodology; confirmation by Public Health Lab reference methodology to follow.” • Send to PHOL to confirm
POS	Neg	<ul style="list-style-type: none"> • Repeat serology to confirm • Repeatable, set up Vitek GNI <p>Shigella spp ID:</p> <ul style="list-style-type: none"> • Release <i>Shigella species</i> with isolate comment \shcn “presumptive isolated. NOTE: Occasionally, some E. coli may identify as presumptive Shigella species using our current in-lab methodology; confirmation by Public Health Lab reference methodology to follow.” • Send to PHOL to confirm <p>NOT a Shigella spp ID:</p> <ul style="list-style-type: none"> • Send to PHOL for ID as follow: <ul style="list-style-type: none"> ○ Non-stool sites: report GNB ID to follow ○ Stool site: Negative test comment add “confirmation by PHOL to follow” <ul style="list-style-type: none"> ▪ do not finalize.



8. Any enteric pathogens identified by Vitek-MS require additional testing

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MS Organism Identification	Additional Testing
<i>Salmonella species</i> * <i>Salmonella species</i> not <i>S. typhi</i> , not <i>S. paratyphi</i> * <i>Salmonella paratyphi</i> A* <i>Salmonella typhi</i> *	Perform serotyping from BA subculture (same day if possible)
NLF <i>E. coli</i> → possible <i>Shigella species</i> *	If Wellcolex Shigella Latex positive, test with Remel Shigella serology . If positive for both serology tests report presumptive ID, then set up Vitek GNI (same day if possible)
NSF <i>E. coli</i> → possible O157*	Subculture to BA for: Oxoid E.coli O157 Latex (same day if possible)
<i>Campylobacter C. jejuni</i> or <i>C. coli</i>	Gram stain if atypical colony.
<i>Yersinia</i> ^{1,2} (any species)	N/A
<i>Vibrio</i> ^{1,2} (any species)	Gram stain Subculture to BA for Oxidase(+)
<i>Plesiomonas</i> ^{1,2} or <i>Aeromonas</i> ^{1,2} (any species)	N/A

¹Send presumptive identification result when additional testing is completed and send all enteric pathogens to PHL to confirm identification except *C. jejuni* or *C. coli*

²If identified by routine specimen processing, proceed to report isolate despite the fact no request for isolation was received.

9. Any *Streptococcus pneumoniae* identified by MALDI >98% require confirmation.
 - 99.9% *S. pneumoniae* by MS that are bile soluble and/or optochin sensitive are confirmed and do not require further work.
 - 98-99.8% *S. pneumoniae* ID by MS that are bile insoluble and optochin resistant are sent out as presumptive *S. viridans* and send to NML for confirmation.
 - Call microbiologist for isolates needing to go to NML for rpoB sequencing (contact Irene Martin at NML - Irene.martin@phac-aspc.gc.ca)



See below for complete algorithm:

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If MALDI 99.9% *S.pneumoniae*

Bile solubility	Optochin	Reporting	Send to NML for rpoB sequencing?
Soluble And/Or	Sensitive	<i>S.pneumoniae</i>	No
Soluble	Resistant	presumptive <i>S.pneumoniae</i>	Yes
Insoluble	Sensitive	presumptive <i>S.pneumoniae</i>	Yes
Insoluble	Resistant	presumptive <i>S.pneumoniae</i>	Yes

If MALDI 98-99.8% *S.pneumoniae*

Bile solubility	Optochin	Reporting	Send to NML to Confirm?
Soluble And/Or	Sensitive	presumptive <i>S.pneumoniae</i>	Yes
Soluble	Resistant	presumptive <i>S.pneumoniae</i>	Yes
Insoluble	Sensitive	presumptive <i>S.pneumoniae</i>	Yes
Insoluble	Resistant	presumptive <i>Viridans strep.</i>	Yes

References: Q3232098 & Q3063546

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10. The following *Neisseria species* specimens must be confirmed by [[NEISSERIA & HAEMOPHILUS \(API NH\)](#)] before reporting.

- *Neisseria species* - Sterile sites
- *N. gonorrhoeae* - ALL sites
- *N. meningitidis* - ALL sites

If a matching ID is not obtained by API (discrepant result), report isolate as follows:



MS ID	API ID	Site	Reporting if discrepant result	Verify in LIS?
<i>Neisseria species</i>	<i>N.meningitidis</i>	Sterile sites	<i>Neisseria, NOS</i> further report to follow	YES
<i>Neisseria species</i>	<i>N.gonorrhoeae</i>	Sterile sites	<i>Neisseria, NOS</i> further report to follow	YES
<i>N. gonorrhoeae</i>	Non- <i>N.gonorrhoeae</i>	Sterile sites	<i>Neisseria, NOS</i> further report to follow	YES
	Non- <i>N.gonorrhoeae</i>	Non-Sterile sites	<i>Neisseria gonorrhoeae</i> (do not release)	NO
<i>N. meningitidis</i>	Non- <i>N.meningitidis</i>	All sites	<i>Neisseria, NOS</i> further report to follow	YES

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Successful Vitek MS identification Tips

The following tips will help achieve successful identification for spots made and create an efficient workflow.

1. Less is more: ensure a **uniformly thin** layer is applied for successful readings.
2. Cover entire spot: having the laser read protein rather than a blank area will shorten the time required reading each spot.
3. BA is preferable: use a colony from blood agar where possible to spot rather than pigmented colonies (e.g. MAC)
4. Use film growth for BC: an early film growth may be used for sterile sites such as blood.
5. Do NOT re-fire red spots: for any red spots, do not re-fire. Alternatively, re-spot and consider using formic acid.
6. Consider FA for mucoid colonies: For mucoid colonies not achieving a successful ID, consider using formic acid.

Vitek MS Etiquette

Setting up runs:

- Remember to record the slide # on the MALDI worksheet
- Match the isolate # with the number on the MALDI worksheet
 - Do not use the same isolate number twice once it has already been **transferred**
- Perform smaller batches and more frequent loads
- Use different prep stations when batching slides
- Leave a MALDI worksheet by the Vitek MS if slide is in progress

Analyzing runs:


- Review your MALDI results soon after it's completed its run
 - Do not leave for extended periods of time without reviewing in MYLA
 - If you will be gone for an extended period, find a technologist willing to review for you

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- Remember to remove your MALDI worksheet when finished with Vitek MS
- Trash all unwanted IDs after each run
- Trash finished worksheets when complete
- Place finished worksheets in the save basket when complete
- Place worksheet for “partially” used MS slides in the “Partial” basket near the machine that was used

Finished runs:

- Notify the following bench when your run is complete (re-fires completed)
- Ensure the colonial morphology is described for the isolate put into the Vitek MS
 - T:test → J: Morph → pick
- For transferred isolates that are not being reported, switch to as corresponding letter (1=A, 2=B..) and **verify isolate.**
- Ensure the MALDI ID correspond/match the morphology/gram stain of the sample

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Vitek MS Quality Control

Each Vitek MS requires a positive and negative control QC performed upon receipt and new lot as well as once daily for each microorganism type. One bacterial and one yeast isolate will be used for positive quality control. A spot with matrix alone will be used for the negative control.

The following quality control strains are to be used on each day of testing with the expected results. The percent confidence achieved will be documented in MicQC.

VITEK MS DAILY QUALITY CONTROL	
QC ORGANISMS	EXPECTED RESULTS
<i>Enterobacter aerogenes</i> 13048	<i>Enterobacter aerogenes</i> ²
<i>Candida glabrata</i> MYA-2950	<i>Candida glabrata</i> ²
No organisms (matrix only)	No ID ¹

¹ If the negative control gives an identification result, visually check the surface of the MS slide to ensure it is clean and repeat negative control

² Repeated low confidence identification following proper procedures and technique may indicate calibration may be required.

If unexpected results are achieved follow [Out of Range Results procedure](#) in the Quality Control Manual.

Vitek MS Maintenance

Routine maintenance is required as scheduled below. All results are documented in the LIS.

Weekly Maintenance

Desiccant Check

Check colour of desiccant is orange. If desiccant is changing colour to pink, change the desiccant.

Area Cleaning

Clean dusk from Vitek MS area including acquisition station and monitor.

Vitek MS Verification and Fine-tuning



When verification/fine-tuning is being performed ensure to place signs on BOTH Vitek MS Machines.

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For machine undergoing Verification/Fine-tuned post with sign:

MONITORING OF THIS INSTRUMENT WILL BE PERFORMED FIRST THING
THIS MORNING
PLEASE LOAD SLIDES IN MACHINE #1 / #2, UNTIL FURTHER NOTICE

For the machine not undergoing Verification/ Fine-tuning post with sign:

THE OTHER MALDI MACHINE IS BEING MONITORED THIS MORNING.
PLEASE ENSURE:
BLOOD CULTURES & INFECTION CONTROL HAVE FIRST PRIORITY FOR
LOADING

Document verification and fine tuning in SoftMic weekly.

Verification:

The calibration status of all instruments are continuously monitored by technical support. A technical support representative will contact the laboratory when an instrument is drifting and verification is required.

To perform verification:



- 1) Prepare **two** complete acquisition groups (32 spots plus the control spots) of a new Vitek MS slide from a fresh *E. coli* ATCC 8739 plate.
- 2) Scan the slide and enter “Ecoli8739” into each of the 32 positions on the slide at the Prep Station
- 3) Load the slide into position 1 (first slot left hand side) in the VITEK MS instrument to be verified (i.e. Instrument 1 or 2). The slide should be placed in the instrument for 8:00am-8:15am maximum in order to respect the arranged schedule
- 4) Start the run, complete the form for the Vitek MS in question located in T:\microbiology\Vitek MS and email to bioMérieux to assess the spots remotely
- 5) If the quality of the spots indicates that a fine tuning is required (determined by bioMérieux technical support), the technical support person will proceed to fine tune the instrument using the same slide
- 6) If the original spots are not of sufficient quality to allow tuning, bioMérieux will be a request the preparation of two further acquisition groups (i.e. 32 spots plus controls)

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- 7) If the verification values are good (as deemed by technical support personnel), technical support will notify us that there is no need to fine tune the instrument.
- 8) To prevent unnecessary spot failures due to instrument drift, monitoring could be scheduled every two weeks, depending on the rate of instrument use.
- 9) Biomérieux hotline team will reply to us through email when the verification/fine-tuning is finalized and to mention:
 - Verification or Fine-tuning needed
 - System ready to go

*Note: If at any time the control fails or the instrument seems to be having an inordinate amount of unidentified isolates, and unscheduled verification may be necessary.

Fine-tuning:

- 1) Prepare a new Vitek MS slide with two full acquisition groups of E. coli 8739
- 2) This time, do not enter scan the slide or enter the spots at the Prep Station
- 3) Merely load the slide into the instrument to be fine tuned notify the technical support person that it has been loaded
- 4) Do NOT start the run
- 5) Technical support will fine tune the instrument remotely and will contact us when it is complete and ready for use
- 6) Fine-tuning date must be documented.


Monthly Maintenance

Adapter Cleaning

Clean adapter with kimwipe.

O ring and Seal Check

Open the door and check the condition of both the O-ring and sealing face of the front door. Wipe away moderate soiling with a clean kimwipe. If badly contaminated, use a alcohol to help clean the door seal.

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Yearly Maintenance

Yearly Maintenance is performed by Vitek MS technical staff. A PM visit must be arranged annually.

Related Documents:

Document name	Document code
Vitek MS Worksheet	
Vitek MS Worksheet with Controls	
VITEK-MS-V2-speciesList Created-Translated list	Policy: #MI\MSID

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Manual Section Name: Vitek MS MALDI-TOF Manual



Page Number / Item	Date of Revision	Signature of Approval
Updated workflow	November 04, 2013	Dr. T. Mazzulli
Updated MALDI acceptance guide	November 19, 2013	Dr. T. Mazzulli
Monitoring and Fine tuning procedure added	December 16, 2013	Dr. T. Mazzulli
If \geq 98%, write the result on the MS worksheet. Put a checkmark if 99.9%. Update some process	December 16, 2013	Dr. T. Mazzulli
Annual Review Date	March 31, 2014	Dr. T. Mazzulli
Fix hyperlink in TOC: MALDI Organism Identification Acceptance. Modified header	June 6, 2014	Dr. T. Mazzulli
Removed duplicate steps 31- 63 from Myla Added Vitek MS etiquette section To Vitek MS monitoring and fine tuning added “Monitoring” paragraph at top of section, and “Note” with 4 bullets	June 10, 2015	Dr. T. Mazzulli
-Updated monitoring procedure to verification procedure. -#3 verification: verification slide to be put at 8am #4 added link to email to hotline for verification/finetuning -Removed testing with strep isolate if maldi has an inordinate amount of unidentified isolates. Added point #10 hotline team reply via email	July 16, 2015	Dr. T. Mazzulli
Beginning of Section: Vitek MS Verification and Fine-tuning added instructions for posting signage on both Vitek MS Machines.	September 17, 2015	Dr. T. Mazzulli
Under Organism acceptance added: For Beta hemolytic strep: GAS, GCS, GGS identified by MALDI, confirmation by Streptococcus Latex Agglutination is needed before reporting.	October 1, 2015	Dr. T. Mazzulli
Under verifications: Added separate email templates for Vitek MS 1 and Vitek MS 2	October 7, 2015	Dr. T. Mazzulli
MS organism ID acceptance added #7 criteria for <i>Streptococcus pneumoniae</i>	November 30, 2015	Dr. T. Mazzulli
Added to organism ID list: always send for confirmation	February 22, 2016	Dr. T. Mazzulli

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as per Biomerieux recall Feb 22 nd 2016 for: <i>Yersinia pestis, Bacillus anthraxis</i>		
Added to Maldi Etiquette: <ul style="list-style-type: none"> Place finished worksheets in the save basket when complete Place worksheet for “partially” used MS slides in the “Partial” basket near the machine that was used 	April 12, 2016	Dr. T. Mazzulli
Removed monitoring values for indication of fine tuning. Document verification and fine tuning in SoftMic weekly. Added Maintenance section with weekly and monthly and yearly maintenance required.	May 16, 2016	Dr. T. Mazzulli
Annual Review Added Related Documents table with link to Vitek myla ID table and MS worksheet	June 10, 2016	Dr. T. Mazzulli
Removed Total Peaks Value communicated and documentation needed. (due to Myla upgrade, no longer required)	December 6, 2016	Dr. T. Mazzulli
Maldi verification spots increased from 16 to 32 spots with controls.	March 13, 2017	Dr. T. Mazzulli
Updated requirements on confirming ID of <i>S.pneumoniae</i> to include performing optochin.	April 6, 2017	Dr. T. Mazzulli
Annual Review	June 10, 2017	Dr. T. Mazzulli
Addition of daily QC section including yeast control.	July 4, 2017	Dr. T. Mazzulli
Addition of expanded Neisseria acceptance rules based on species and sites. Added enteric media additional testing Expanded procedure for NLF <i>E.coli</i> .	August 15, 2017	Dr. T. Mazzulli
Annual Review Updated link to location verification email to technical support. Added section :Tips for successful identification	April 26, 2018	Dr. T. Mazzulli

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