

PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC60070 – Stock Culture Maintenance	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved:
Accreditation Canada Applicable Standard: NA	

Uncontrolled When Printed

GUIDING PRINCIPLE:

Reference strains for quality control are originally obtained from commercial sources as lyophilized cultures. Following the manufacturer's instructions, the lyophilized cultures will be subbed out to the appropriate media on the required schedule and stored in beads containing trisodium citrate glycerol at -70°C. These frozen cultures are used as STOCK CULTURES and can be stored for approximately one year.

PURPOSE/RATIONALE:

Stock cultures of ATCC quality control organisms are sub cultured according to a schedule in order to maintain optimum performance.

SCOPE/APPLICABILITY:

This procedure applies to Medical Laboratory Technologists (MLTs) performing quality control testing in the microbiology laboratory.

REAGENTS and/or MEDIA:

- Blood agar (BA)
- Chocolate agar (CHO)
- Nutrient slant (NUT)
- Blood slant (BASLT)

SUPPLIES:

- Disposable inoculation needles
- Pro-lab Microbank vials
- KWIK-STIKs

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EQUIPMENT:

- Ultra-low freezer
- Biosafety cabinet
- Disposable inoculation needles
- 35° CO₂ incubators

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

PROCEDURE INSTRUCTIONS:

Step	Action
KWIK-STIK Instructions:	
1	MicroBiologics KWIK-STIKs are ordered yearly from Micronstyx.
2	Acquire new lyophilized organisms from the Microbiology reagent fridge.
3	Allow the unopened KWIK-STIK pouch to equilibrate to room temperature. Tear open pouch at notch and remove the KWIK-STIK unit.
4	Tear off pull-tab portion on the label and attach it to the primary culture plate. Do not disassemble the device during hydration.
5	Pinch (once only) the ampoule at the top of the KWIK-STIK in the cap to release the hydrating fluid.
6	Hold vertically and tap on a hard surface to facilitate flow of fluid through shaft to the pellet into the bottom portion of the unit containing the pellet.
7	Using a pinching action on the bottom portion of the unit, crush the pellet in the fluid until the pellet suspension is homogenous.
8	Immediately heavily saturate the swab with the hydrated material and transfer to agar. Inoculate the culture plate by gently rolling the swab over one-third of the plate. Using a sterile loop, streak to facilitate colony isolation.
9	Immediately incubate the inoculated primary culture plate in the CO ₂ incubator.
10	The next day, the organism will need to be sub cultured a second time to the appropriate agar before inoculating the microbank vial.

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Step	Action
Microbank Instructions:	
1	QC organisms are stored in Microbank vials, which offers a platform that utilizes porous glass beads and a specially formulated cryo-preserved for storage at low temperatures. The vials are stored at -80°C
2	Label vial with organism's TQC label. Ensure to use the active lot number label. Place a piece of packing tape over the label to protect it from moisture
3	In the BSC, using aseptic technique, unscrew the Microbank vial cap. Using a sterile inoculating loop pick off enough colonies from a pure culture to achieve a 3-4 McFarland standard in the cryo-preserved
4	Using aseptic technique, replace the cap on the Microbank vial tightly and invert it 4-5 times to emulsify the organism. DO NOT VORTEX.
5	Let the Microbank vial sit for 2 minutes to allow the isolate to bind to the beads. Remove the cap and use a sterile pipette to remove the cryo-preserved. The beads should be as free of liquid as possible.
6	Close the Microbank vial finger tight only. It is important that the Microbank vials are not overtightened.

Step	Action																																		
Yearly Stock Culture Maintenance Instructions:																																			
1	QC organisms are sub cultured yearly, from the KWIK STIK lyophilized organisms and stored into Microbank vials. Yearly sub culturing of QC organisms is to be performed in August of each year																																		
2	Follow the instructions above for how to use the KWIK STIK unit.																																		
3	Follow the instructions above for how to transfer organisms to Microbank vials.																																		
4	<p>The following is a list of the organisms that are frozen yearly:</p> <table> <tr> <td>✓ <i>E. faecalis</i> 29212</td><td>✓ <i>P. aeruginosa</i> 27853</td></tr> <tr> <td>✓ <i>E. faecalis</i> 51299</td><td>✓ <i>S. pneumoniae</i> 49619</td></tr> <tr> <td>✓ <i>E. faecium</i> 700221</td><td>✓ <i>H. influenzae</i> 10211</td></tr> <tr> <td>✓ <i>S. aureus</i> 29213</td><td>✓ <i>H. influenzae</i> 49247</td></tr> <tr> <td>✓ <i>S. aureus</i> 25923</td><td>✓ <i>H. influenzae</i> 49766</td></tr> <tr> <td>✓ <i>S. aureus</i> 43300</td><td>✓ <i>C. albicans</i> 14053</td></tr> <tr> <td>✓ <i>S. aureus</i> BAA-977</td><td>✓ <i>C. glabrata</i> 66032</td></tr> <tr> <td>✓ <i>S. aureus</i> BAA-1026</td><td>✓ <i>C. tropicalis</i> 750</td></tr> <tr> <td>✓ <i>S. agalactiae</i> 12386</td><td>✓ <i>C. krusei</i> 14243</td></tr> <tr> <td>✓ <i>S. agalactiae</i> 13813</td><td>✓ <i>C. parapsilosis</i> 90018</td></tr> <tr> <td>✓ <i>S. pyogenes</i> 19615</td><td>✓ <i>S. saprophyticus</i> BAA-750</td></tr> <tr> <td>✓ <i>S. epidermidis</i> 12228</td><td>✓ <i>E. casseliflavus</i> 700327</td></tr> <tr> <td>✓ <i>E. coli</i> 25922</td><td>✓ <i>S. maltophilia</i> 17666</td></tr> <tr> <td>✓ <i>E. coli</i> 35218</td><td>✓ <i>E. hormaechei</i> 700323</td></tr> <tr> <td>✓ <i>K. pneumoniae</i> 700603</td><td>✓ <i>E. corrodens</i> BAA-1152</td></tr> <tr> <td>✓ <i>P. mirabilis</i> 7002</td><td>✓ <i>C. septicum</i> 12464</td></tr> <tr> <td></td><td>✓ <i>B. ovatus</i> BAA-1296</td></tr> </table>	✓ <i>E. faecalis</i> 29212	✓ <i>P. aeruginosa</i> 27853	✓ <i>E. faecalis</i> 51299	✓ <i>S. pneumoniae</i> 49619	✓ <i>E. faecium</i> 700221	✓ <i>H. influenzae</i> 10211	✓ <i>S. aureus</i> 29213	✓ <i>H. influenzae</i> 49247	✓ <i>S. aureus</i> 25923	✓ <i>H. influenzae</i> 49766	✓ <i>S. aureus</i> 43300	✓ <i>C. albicans</i> 14053	✓ <i>S. aureus</i> BAA-977	✓ <i>C. glabrata</i> 66032	✓ <i>S. aureus</i> BAA-1026	✓ <i>C. tropicalis</i> 750	✓ <i>S. agalactiae</i> 12386	✓ <i>C. krusei</i> 14243	✓ <i>S. agalactiae</i> 13813	✓ <i>C. parapsilosis</i> 90018	✓ <i>S. pyogenes</i> 19615	✓ <i>S. saprophyticus</i> BAA-750	✓ <i>S. epidermidis</i> 12228	✓ <i>E. casseliflavus</i> 700327	✓ <i>E. coli</i> 25922	✓ <i>S. maltophilia</i> 17666	✓ <i>E. coli</i> 35218	✓ <i>E. hormaechei</i> 700323	✓ <i>K. pneumoniae</i> 700603	✓ <i>E. corrodens</i> BAA-1152	✓ <i>P. mirabilis</i> 7002	✓ <i>C. septicum</i> 12464		✓ <i>B. ovatus</i> BAA-1296
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Monthly Stock Culture Maintenance Instructions:																			
1	QC organisms are sub cultured monthly, from the Microbank vials, to be used for daily, weekly and as-needed quality control testing.																		
2	Monthly sub culturing of QC organisms is to be performed on the first Monday of the month.																		
3	Remove the Microbank vial from the ultra-low freezer.																		
4	Label appropriate media with QC organisms' identification using the labels located in the "Microbiology QC Stickers" binder.																		
5	In the BSC, using aseptic technique, open the Microbank vial and use a sterile needle to remove one coloured bead. Close the Microbank vial finger tight and return to the freezer as soon as possible. NOTE: Excessive changes in temperature will reduce the viability of the frozen isolates.																		
6	The bead may then be streaked directly onto a solid medium. Streak for isolated growth.																		
7	<p>The following is a list of the non-fastidious organisms that are sub-cultured monthly from the frozen beads on Monday to Blood agar:</p> <table border="0"> <tr> <td>✓ <i>E. faecalis</i> 29212</td><td>✓ <i>S. aureus</i> 43300</td></tr> <tr> <td>✓ <i>E. faecalis</i> 51299</td><td>✓ <i>S. aureus</i> BAA-977</td></tr> <tr> <td>✓ <i>E. faecium</i> 700221</td><td>✓ <i>S. aureus</i> BAA-1026</td></tr> <tr> <td>✓ <i>S. agalactiae</i> 12386</td><td>✓ <i>E. coli</i> 25922</td></tr> <tr> <td>✓ <i>S. agalactiae</i> 13813</td><td>✓ <i>E. coli</i> 35218</td></tr> <tr> <td>✓ <i>S. pyogenes</i> 19615</td><td>✓ <i>K. pneumoniae</i> 700603</td></tr> <tr> <td>✓ <i>S. epidermidis</i> 12228</td><td>✓ <i>P. mirabilis</i> 7002</td></tr> <tr> <td>✓ <i>S. aureus</i> 29213</td><td>✓ <i>P. aeruginosa</i> 27853</td></tr> <tr> <td>✓ <i>S. aureus</i> 25923</td><td></td></tr> </table> <ul style="list-style-type: none"> On Tuesday, subculture each organism to a new BA plate and the assigned slant as per MIC60071-Stock Culture Maintenance Job Aid <ul style="list-style-type: none"> ➤ Incubate the plates and slants in the CO₂ incubator at 35° overnight On Wednesday, use the second BA plate to perform the daily and weekly QC testing <ul style="list-style-type: none"> ➤ Store the slants in the microbiology specimen fridge ➤ Store daily QC plates in the microbiology specimen fridge ➤ Store weekly QC plates in the CO₂ incubator ➤ Discard the previous week's slants and QC plates 	✓ <i>E. faecalis</i> 29212	✓ <i>S. aureus</i> 43300	✓ <i>E. faecalis</i> 51299	✓ <i>S. aureus</i> BAA-977	✓ <i>E. faecium</i> 700221	✓ <i>S. aureus</i> BAA-1026	✓ <i>S. agalactiae</i> 12386	✓ <i>E. coli</i> 25922	✓ <i>S. agalactiae</i> 13813	✓ <i>E. coli</i> 35218	✓ <i>S. pyogenes</i> 19615	✓ <i>K. pneumoniae</i> 700603	✓ <i>S. epidermidis</i> 12228	✓ <i>P. mirabilis</i> 7002	✓ <i>S. aureus</i> 29213	✓ <i>P. aeruginosa</i> 27853	✓ <i>S. aureus</i> 25923	
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2	<p>The following is a list of the fastidious organisms that are sub-cultured weekly from the previous week's BA QC plates on Tuesday to Blood agar:</p> <ul style="list-style-type: none"> ✓ <i>S. pneumoniae</i> 49619 On Wednesday, use the second BA plate to perform the weekly QC testing <ul style="list-style-type: none"> ➤ Store weekly QC plates in the CO₂ incubator ➤ Discard the previous week's QC plates 																		
3	<p>The following is a list of the fastidious organisms that are sub-cultured weekly from the previous week's CHO QC plates on Tuesday to Choc agar:</p> <table border="0"> <tr> <td>✓ <i>H. influenzae</i> 10211</td><td>✓ <i>C. glabrata</i> 66032</td></tr> <tr> <td>✓ <i>H. influenzae</i> 49247</td><td>✓ <i>C. tropicalis</i> 750</td></tr> <tr> <td>✓ <i>H. influenzae</i> 49766</td><td>✓ <i>C. krusei</i> 14243</td></tr> <tr> <td>✓ <i>C. albicans</i> 14053</td><td>✓ <i>C. parapsilosis</i> 22019</td></tr> </table> <ul style="list-style-type: none"> On Wednesday, use the second CHO plate to perform the weekly QC testing <ul style="list-style-type: none"> ➤ Store weekly QC plates in the CO₂ incubator ➤ Discard the previous week's QC plates 	✓ <i>H. influenzae</i> 10211	✓ <i>C. glabrata</i> 66032	✓ <i>H. influenzae</i> 49247	✓ <i>C. tropicalis</i> 750	✓ <i>H. influenzae</i> 49766	✓ <i>C. krusei</i> 14243	✓ <i>C. albicans</i> 14053	✓ <i>C. parapsilosis</i> 22019										
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Step	Action
As required Stock Culture Maintenance Instructions	
1	<p><u>The following is a list of the non-fastidious organisms that are sub-cultured as required from the frozen beads to Blood agar for VITEK 2 GNI card QC:</u></p> <ul style="list-style-type: none"> ✓ <i>Enterobacter hormaechei</i> ATCC 700323 ✓ <i>Stenotrophomonas maltophilia</i> ATCC 17666 <ul style="list-style-type: none"> • On the second day, subculture each organism to a new BA plate <ul style="list-style-type: none"> ➤ Incubate the plates in the CO₂ incubator at 35° overnight • On the third day, use the second BA plate to perform the VITEK 2 GNI card QC • After QC is performed, store plates in the CO₂ incubator • Discard plates after QC testing is complete and acceptable
2	<p><u>The following is a list of the non-fastidious organisms that are sub-cultured as required from the frozen beads to Blood agar and TSA slant for VITEK 2 GPI card QC:</u></p> <ul style="list-style-type: none"> ✓ <i>Staphylococcus saprophyticus</i> ATCC BAA-750 ✓ <i>Enterococcus casseliflavus</i> ATCC 700327 <ul style="list-style-type: none"> ➤ Subculture <i>Staphylococcus saprophyticus</i> to a BA plate and incubate plates in CO₂ incubator at 35° overnight ➤ Subculture <i>Enterococcus casseliflavus</i> to a TSA slant and incubate in CO₂ incubator at 35° overnight • On the second day, subculture each organism a second time: <ul style="list-style-type: none"> ➤ Subculture <i>Staphylococcus saprophyticus</i> to a BA plate and incubate in CO₂ incubator at 35° overnight ➤ Subculture <i>Enterococcus casseliflavus</i> to TSA slant and incubate in CO₂ incubator at 35° overnight • On the third day, use the second BA plate and the second BA slant to perform the VITEK 2 GPI card QC • After QC is performed, store plate and slant in the CO₂ incubator • Discard plate and slant after QC testing is complete and acceptable
3	<p><u>The following is the fastidious organism that is sub-cultured as required from the frozen beads to Chocolate agar VITEK 2 NH card QC:</u></p> <ul style="list-style-type: none"> ✓ <i>Eikenella corrodens</i> ATCC BAA-1152 <ul style="list-style-type: none"> • On the second day, subculture organism to a new CHO plate <ul style="list-style-type: none"> ➤ Incubate the plate in the CO₂ incubator at 35° overnight • On the third day, use the second CHO plate to perform the VITEK 2 NH card QC • After QC is performed, store plate in the CO₂ incubator • Discard plate after QC testing is complete and acceptable

4	<p>The following is a list of the anaerobic organisms that are sub-cultured as required from the frozen beads to Brucella agar for VITEK 2 ANC card QC:</p> <ul style="list-style-type: none">✓ <i>Clostridium septicum</i> ATCC12464✓ <i>Bacterioides ovatus</i> ATCC BAA-1296• On the third day, subculture organisms to new BRU plate<ul style="list-style-type: none">➤ Incubate anaerobically for 48 hours• On the fifth day, use the second BRU plate to perform the VITEK 2 ANC card QC• After QC is performed, store plates anaerobically• Discard plates after QC testing is complete and acceptable
5	<p>The following is the non-fastidious organism that is sub-cultured as required from the frozen beads to Blood agar for API 20 E QC:</p> <ul style="list-style-type: none">✓ <i>Proteus mirabilis</i> ATCC 35659• On the second day, subculture organism to new BA plate<ul style="list-style-type: none">➤ Incubate the plate in the CO₂ incubator at 35° overnight• On the third day, use the second BA plate to perform the API 20 E QC• After QC is performed, store plate in the CO₂ incubator• Discard plate after QC testing is complete and acceptable
6	<p>The following is the fastidious organism that is sub-cultured as required from the KWIK STIK to Chocolate agar for API NH QC:</p> <ul style="list-style-type: none">✓ <i>Neisseria gonorrhoeae</i> ATCC 31426• On the second day, subculture organism to a new CHO plate• On the third day, use the second CHO plate to perform the API NH QC• After QC is performed, store plate in the CO₂ incubator• Discard plate after QC testing is complete and acceptable

CROSS REFERENCES:

NA

REFERENCES:

- CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 35th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2025
- MicroBiologics *KWIK STIK* package insert, 2022.MAR.10
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APPROVAL:

Date

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	15 Sep 17	Initial Release	L. Steven
2.0	06 Oct 19	Procedure reviewed	L. Steven
3.0	05 Jul 21	Procedure reviewed and added to NTHSSA policy template	L. Steven
4.0	03 Jul 23	Procedure reviewed	L. Steven
5.0	28 Apr 25	Procedure reviewed	L. Steven

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