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, Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
Reference strains for quality control are originally obtained from commercial sources as lyophilised 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 -70° freezer
- Biosafety cabinet
- Disposable inoculation needles
- 35° ambient air and 35° CO_2 incubators

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential 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-STIXs 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. Allow the hydrating fluid to flow through the swab shaft and 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 at temperature and conditions appropriate to the microorganism.

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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-preservative 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-preservative
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-preservative. 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 organism 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	The following is a list of the organisms that are frozen yearly: ✓ ***E.faecalis* ATCC 29212** ✓ ***E.faecalis* ATCC 51299** ✓ ***E.faecium* ATCC 700221** ✓ ***S.aureus* ATCC 29213** ✓ ***S.aureus* ATCC 25923** ✓ ***S.aureus* ATCC 43300** ✓ ***S.aureus* ATCC BAA-977** ✓ ***S.aureus* ATCC BAA-1026** ✓ ***S.agalactiae* ATCC 12386** ✓ ***S.agalactiae* ATCC 13813** ✓ ***S.pyogenes* ATCC 19615** ✓ ***S.epidermidis* ATCC 12228** ✓ ***E.coli* ATCC 25922** ✓ ***E.coli* ATCC 35218** ✓ ***K.pneumoniae* ATCC 700603** ✓ ***P.mirabilis* ATCC 7002** ✓ ***P.aeruginosa* ATCC 27853** ✓ ***A.aphrophilus* ATCC 7901 (CHO, CO_2)** ✓ ***H.influenzae* ATCC 10211 (CHO, CO_2)** ✓ ***H.influenzae* ATCC 49247 (CHO, CO_2)** ✓ ***H.influenzae* ATCC 49766 (CHO, CO_2)** ✓ ***M.catarrhalis* ATCC 25240** ✓ ***S.pneumoniae* ATCC 49619** ✓ ***S.salivarius* ATCC 13419** ✓ ***S.saprophyticus* ATCC BAA-750** ✓ ***E.casseliflavus* ATCC 700327** ✓ ***S.maltophilia* ATCC 17666** ✓ ***Enterobacter hormaechei* ATCC 700323**

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Step	Action
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 by the urine bench technologist.
3	Remove the Microbank vial from the -70°C freezer.
4	Label appropriate media with QC organisms' identification using the labels located in the "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. 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	<u>The following is a list of the non-fastidious organisms that are sub-cultured monthly from the frozen beads on Monday to Blood agar:</u> ✓ ***E.faecalis* ATCC 29212** ✓ ***E.faecalis* ATCC 51299** ✓ ***E.faecium* ATCC 700221** ✓ ***S.aureus* ATCC 29213** ✓ ***S.aureus* ATCC 25923** ✓ ***S.aureus* ATCC 43300** ✓ ***S.aureus* ATCC BAA-977** ✓ ***S.aureus* ATCC BAA-1026** ✓ ***S.agalactiae* ATCC 12386** ✓ ***S.agalactiae* ATCC 13813** ✓ ***S.pyogenes* ATCC 19615** ✓ ***S.epidermidis* ATCC 12228** ✓ ***E.coli* ATCC 25922** ✓ ***E.coli* ATCC 35218** ✓ ***K.pneumoniae* ATCC 700603** ✓ ***P.mirabilis* ATCC 7002** ✓ ***P.aeruginosa* ATCC 27853** • On Tuesday, subculture each organism to a new Blood agar plate and the assigned slant as per MIC60071-Stock Culture Maintenance Job Aid ➢ Incubate the plates and slants in the CO_2 incubator at 35° overnight • On Wednesday, use the second BA sub-culture plate to perform the daily and weekly QC testing ➢ Store the slants in the microbiology specimen fridge ➢ Store daily QC plates in the blue rack ➢ Store weekly QC plates in the blue bucket in the CO_2 incubator ➢ Discard the previous weeks slants and QC plates
8	<u>The following is a list of the fastidious organisms that are sub-cultured monthly from the frozen beads on Monday to Blood agar</u>: ✓ ***M.catarrhalis* ATCC 25240** ✓ ***S.pneumoniae* ATCC 49619** ✓ ***S.salivarius* ATCC 13419** • On Tuesday, subculture each organism to a new Blood agar plate ➢ Incubate the plates in the CO_2 incubator at 35° overnight • On Wednesday, use the second BA sub-culture plate to perform the weekly QC testing ➢ Store weekly QC plates in the blue bucket in the CO_2 incubator ➢ Discard the previous weeks QC plates

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9	<u>The following is a list of the fastidious organisms that are sub-cultured monthly from the frozen beads on Monday to Chocolate agar</u>: ✓ ***A.aphrophilus*** **ATCC 7901** ✓ ***H.influenzae*** **ATCC 10211** ✓ **H.influenzae ATCC 49247** ✓ **H.influenzae ATCC 49766** • On Tuesday, subculture to new Chocolate agar plate ➢ Incubate the plates in the CO_2 incubator at 35° overnight • On Wednesday, use the second Chocolate plate to perform the weekly QC testing ➢ Store weekly QC plates in the blue bucket in the CO_2 incubator ➢ Discard the previous weeks QC plates

Step	Action
Weekly Stock Culture Maintenance Instructions:	
1	<u>The following is a list of the non-fastidious organisms that are sub-cultured weekly from the slants on Tuesday to Blood agar:</u> ✓ ***E.faecalis*** **ATCC 29212** ✓ ***E.faecalis*** **ATCC 51299** ✓ ***E.faecium*** **ATCC 700221** ✓ ***S.aureus*** **ATCC 29213** ✓ ***S.aureus*** **ATCC 25923** ✓ ***S.aureus*** **ATCC 43300** ✓ ***S.aureus*** **ATCC BAA-977** ✓ ***S.aureus*** **ATCC BAA-1026** ✓ ***S.agalactiae*** **ATCC 12386** ✓ ***S.agalactiae*** **ATCC 13813** ✓ ***S.pyogenes*** **ATCC 19615** ✓ ***S.epidermidis*** **ATCC 12228** ✓ ***E.coli*** **ATCC 25922** ✓ ***E.coli*** **ATCC 35218** ✓ ***K.pneumoniae*** **ATCC 700603** ✓ ***P.mirabilis*** **ATCC 7002** ✓ ***P.aeruginosa*** **ATCC 27853** • On Wednesday, use the BA sub-culture plate to perform the daily and weekly QC testing ➢ Store daily QC plates in the blue rack ➢ Store weekly QC plates in the blue bucket in the CO_2 incubator ➢ Discard the previous weeks QC plates
9	<u>The following is a list of the fastidious organisms that are sub-cultured weekly from the previous weeks QC BA plates:</u> ✓ ***M.catarrhalis*** **ATCC 25240** ✓ ***S.pneumoniae*** **ATCC 49619** ✓ ***S.salivarius*** **ATCC 13419** • On Wednesday, use the BA sub-culture plate to perform the weekly QC testing ➢ Store weekly QC plates in the blue bucket in the CO_2 incubator ➢ Discard the previous weeks QC plates
10	<u>The following is a list of the fastidious organisms that are sub-cultured monthly from the frozen beads on Monday to Chocolate agar</u>: ✓ ***A.aphrophilus*** **ATCC 7901** ✓ ***H.influenzae*** **ATCC 10211** ✓ **H.influenzae ATCC 49247** ✓ **H.influenzae ATCC 49766** • On Tuesday, subculture to new Chocolate agar plate ➢ Incubate the plates in the CO_2 incubator at 35° overnight • On Wednesday, use the second CHO sub-culture plate to perform the weekly QC testing ➢ Store weekly QC plates in the blue bucket in the CO_2 incubator ➢ Discard the previous weeks QC plates

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Step	Action
As-required Stock Culture Maintenance Instructions	
1	Vitek 2 GNI card organisms stock culture maintenance: • On the first day, subculture the following organisms from the Microbank • Subculture organisms to BA and incubate plates in CO_2 incubator at 35° ✓ ***Enterobacter hormaechei* ATCC 700323** ✓ ***Stenotrophomonas maltophilia* ATCC 17666** • On the second day, subculture each organism to a new BA plate • On the third day, use the second BA subculture plate to perform the Vitek 2 GNI QC testing • After QC is performed, store plates in the CO_2 incubator
2	Vitek 2 GPI card organisms stock culture maintenance: • On the first day, subculture the following organisms from the Microbank • Subculture organisms to BA and incubate plates in CO_2 incubator at 35° ✓ ***Enterococcus casseliflavus* ATCC 700327** ✓ ***Staphylococcus saprophyticus* ATCC BAA-750** • On the second day, subculture each organism to a new BA plate • On the third day, use the second BA subculture plate to perform the Vitek 2 GPI QC testing • After QC is performed, store plates in the CO_2 incubator
3	Vitek 2 NH card organism stock culture maintenance: • On the first day, subculture the following organism from the Microbank • Subculture organism to BA and incubate plate in CO_2 incubator at 35° ✓ ***Eikenella corrodens* ATCC BAA-1152** • On the second day, subculture organism to a new BA plate • On the third day, use the second BA agar subculture plate to perform the Vitek 2 NH QC testing • After QC is performed, store plates in the CO_2 incubator
4	Vitek 2 YST card organism stock culture maintenance: • On the first day, subculture the following organism from the Microbank • Subculture organism to SAB agar and incubate plate at room temperature: ✓ ***Candida albicans* ATCC 14053** • On the second day, subculture organism to a new SAB plate • On the third day, use the second SAB agar subculture plate to perform the Vitek 2 YST QC testing • After QC is performed, store plates at room temperature
5	Vitek 2 ANC card organism stock culture maintenance: • On the first day, subculture the following organisms from the Microbank • Subculture organisms to BRU agar and incubate anaerobically for 48 hours: ✓ ***Clostridium septicum* ATCC12464** ✓ ***Bacterioides ovatus* ATCC BAA-1296** • On the second day, subculture organism to a new BRU agar plate • On the third day, use the second BRU agar subculture plate to perform the Vitek 2 ANC QC testing • After QC is performed, store plates anaerobically

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8	<u>API 20 E organism stock culture maintenance:</u> • On the first day, subculture the following organism from the Microbank • Subculture organism to BA and incubate in CO_2 incubator at 35° ✓ ***Proteus mirabilis* ATCC 35659** • On the second day, subculture organism to a new BA plate • On the third day, use the second BA subculture plate to perform the API 20 E QC testing • After QC is performed, store plates in the CO_2 incubator
9	<u>API NH organism stock culture maintenance</u>: • On the first day, subculture the following organism from the Microbank • Subculture organism to CHO and incubate plate in CO_2 incubator at 35° ✓ ***Neisseria gonorrhoeae* ATCC 31426** • On the second day, subculture organism to a new Chocolate agar plate • On the third day, use the second Chocolate agar subculture plate to perform the API NH QC testing • After QC is performed, store plate in the CO2 incubator

REFERENCES:

- CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.
- MicroBiologics Recommended Growth Requirements Lyfo disc and Kwik-Stik microorganisms package insert, 2017.Jan.17.
- Pro-Lab Diagnostics, Microbank package insert, 2012 11.

APPROVAL:

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Date

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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

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