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

REAGENTS and/or MEDIA:

- Blood agar, Chocolate agar and Sabouraud agar
- Microbiologics KWIK-STIK lyophilized microorganisms
- Microbank cryopreservative solution beads

SUPPLIES:

- Ultra-low -70° freezer
- Biosafety cabinet
- Disposable inoculation needles
- 35° ambient air and 37° CO_2 incubators

SPECIAL SAFETY PRECAUTIONS:

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

- 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. Universal precautions 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:

KWIK-STIK instructions:

- Acquire new lyophilized organisms for all quality control organisms. MicroBiologics KWIK-STIK is used for this purpose and is stored in the reagent refrigerator. Each KWIK-STIK unit contains a lyophilized microorganism pellet, an ampoule of hydrating fluid and an inoculating swab.

KWIK-STIK Microorganism Procedure:

1. Allow the unopened KWIK-STIK pouch to equilibrate to room temperature.
2. Tear open pouch at notch and remove the KWIK-STIK unit.
3. Tear off Pull-Tab portion on the label and attach it to the primary culture plate. Do not disassemble the device during hydration.
4. Pinch (once only) the ampoule at the top of the KWIK-STIK (just below the fluid meniscus of the ampoule) found in the cap to release the hydrating fluid.
5. Hold vertically and tap on a hard surface to facilitate flow of fluid through shaft into bottom of unit containing pellet. Allow the hydrating fluid to flow through the swab shaft and into the bottom portion of the unit containing the pellet.
6. Using a pinching action on the bottom portion of the unit, crush the pellet in the fluid until the pellet suspension is homogenous.
7. Immediately heavily saturate the swab with the hydrated material and transfer to agar.
8. Inoculate the culture plate by gently rolling the swab over one-third of the plate.
9. Using a sterile loop, streak to facilitate colony isolation.
10. Using proper biohazard disposal, discard the KWIK-STIK.
11. Immediately incubate the inoculated primary culture plate at temperature and conditions appropriate to the microorganism.

Microbank instructions:

- QC organisms are stored in Microbank vials, which offers a platform that utilizes porous glass beads and a specially formulated cryopreservative for storage at low temperatures.

To inoculate Microbank vials:

1. Label vial with organism's TQC label. Ensure to use the active lot number label.
2. 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 cryopreservative.
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 cryopreservative. 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.
7. Place the Microbank vial in the Microbank Freezer Storage Box and freeze at -70°C.

Yearly stock culture maintenance instructions:

- 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.
- Follow the instructions on page 2 for how to use the Kwik-Stik unit.
- Follow the instructions on page 2 for how to transfer organisms to Microbank vials.
- The following is a list of the organisms that are frozen yearly:

Campylobacter jejuni ATCC 33291
Enterococcus faecalis ATCC 29212
Enterococcus faecalis ATCC 51299
Enterococcus casseliflavus ATCC 700327
Enterobacter cloacae ATCC 13406
Enterobacter hormaechei ATCC 700323
Escherichia coli ATCC 25922
Escherichia coli ATCC 35218
Escherichia coli O157 ATCC43888
Klebsiella pneumoniae ATCC 700603
Moraxella catarrhalis ATCC 25240
Proteus mirabilis ATCC 7002
Proteus mirabilis ATCC 35659
Pseudomonas aeruginosa ATCC 27853
Salmonella enterica ATCC 14028
Stenotrophomonas maltophilia ATCC 17666
Staphylococcus aureus ATCC 25923
Staphylococcus aureus ATCC 29213
Staphylococcus aureus ATCC 43300
Staphylococcus aureus ATCC BAA-977
Staphylococcus aureus ATCC BAA-1026
Staphylococcus saprophyticus ATCC 15305
Staphylococcus saprophyticus ATCC BAA-750
Staphylococcus epidermidis ATCC 12228
Streptococcus agalactiae ATCC 12386
Streptococcus agalactiae ATCC 13813
Streptococcus pneumoniae ATCC 49619
Streptococcus pyogenes ATCC 19615
Streptococcus salivarius ATCC 13419

Aggregatibacter aphrophilus ATCC 7901
Haemophilus influenzae ATCC 49247
Haemophilus influenzae ATCC 49766
Haemophilus influenzae ATCC 10211
Neisseria gonorrhoeae ATCC 31426
Eikenella corrodens ATCC BAA-1152

Candida albicans ATCC 14053 (SAB, room temperature)
Candida glabrata ATCC 2950 (SAB, room temperature)

Monthly stock culture maintenance instructions

- QC organisms are sub cultured monthly, from the Microbank vials, to be used for daily, weekly and as-needed quality control testing.
- Monthly sub culturing of QC organisms is to be performed on the first Monday of the month by the urine bench technologist.

Recovery of bacterial isolates from Microbank vials:

1. Remove the Microbank vial from the -70°C freezer.
2. Label appropriate media with QC organisms identification using the labels located in the "QC Stickers" binder.
3. In the BSC, using aseptic technique, open the Microbank vial and using a sterile needle to remove one coloured bead. Close the Microbank vial finger tight and return as soon as possible to the freezer. Excessive changes in temperature will reduce the viability of the frozen isolates.
4. The bead may then be streaked directly onto a solid medium. Streak for isolated growth.

Monthly non-fastidious organisms stock culture maintenance:

1. On Monday, subculture the following organisms from the Microbank vials for QC testing. Subculture organisms to Blood agar and incubate plates in CO_2 incubator at 35° overnight:

Enterococcus faecalis ATCC 29212	*Staphylococcus aureus* ATCC 25923
Enterococcus faecalis ATCC 51299	*Staphylococcus aureus* ATCC 29213
Enterobacter cloacae ATCC 13406	*Staphylococcus aureus* ATCC 43300
Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC BAA-977
Escherichia coli ATCC 35218	*Staphylococcus aureus* ATCC BAA-1026
Escherichia coli ATCC 43888	*Staphylococcus epidermidis* ATCC 12228
Klebsiella pneumoniae ATCC 700603	*Staphylococcus saprophyticus* ATCC 15305
Proteus mirabilis ATCC 7002	*Streptococcus agalactiae* ATCC 12386
Pseudomonas aeruginosa ATCC 27853	*Streptococcus agalactiae* ATCC 13813
Salmonella enterica ATCC 14028	*Streptococcus pyogenes* ATCC 19615

2. On Tuesday, subculture each organism to a new Blood agar plate and the assigned slant as per MIC60071. Incubate the plates and slants in the CO_2 incubator at 35° overnight.
3. On Wednesday, use the second Blood agar subculture plate to perform the daily and weekly QC testing and store the slants in the microbiology specimen refrigerator for weekly sub culturing. Discard previous week's slants.
4. After QC is performed, discard previous week's subculture plates and store new plates in red QC plates bucket.

Monthly fastidious (Blood agar) organisms stock culture maintenance:

1. On Monday, subculture the following organisms from the Microbank vials for QC testing. Subculture organisms to Blood agar and incubate plates in CO_2 incubator at 35° overnight:

   Moraxella catarrhalis ATCC 25240
   Streptococcus pneumoniae ATCC 49619
   Streptococcus salivarius ATCC 13419

2. On Tuesday, subculture each organism to a new Blood agar plate. Incubate the plates in the CO_2 incubator at 35° overnight.
3. On Wednesday, use the second Blood agar subculture plate to perform the weekly QC testing.
4. After QC is performed, discard previous week's subculture plates and store new plates in "CO_2 plate rack" in CO_2 incubator.

Monthly fastidious (Chocolate agar) organisms stock culture maintenance:

1. On Monday, subculture the following organisms from the Microbank vials for QC testing. Subculture organisms to Chocolate agar and incubate plates in CO_2 incubator at 35° overnight:

   Aggregatibacter aphrophilus ATCC 7901
   Haemophilus influenzae ATCC 10211
   Haemophilus influenzae ATCC 49247
   Haemophilus influenzae ATCC 49766

2. On Tuesday, subculture each organism to a new Chocolate agar plate. Incubate the plates in CO_2 incubator at 35° overnight.
3. On Wednesday, use the second Chocolate agar subculture plate to perform the weekly QC testing.
4. After QC is performed, discard previous week's subculture plates and store new plates in "CO_2 plate rack" in CO_2 incubator.

Weekly stock culture maintenance instructions

Weekly non-fastidious organisms stock culture maintenance:

1. On Tuesday, subculture the following organisms from the slants for QC testing. Subculture organisms to Blood agar and incubate plates in CO_2 incubator at 35° overnight:

   Enterococcus faecalis ATCC 29212
   Enterococcus faecalis ATCC 51299
   Enterobacter cloacae ATCC 13406
   Escherichia coli ATCC 25922
   Escherichia coli ATCC 35218
   Escherichia coli ATCC 43888
   Klebsiella pneumoniae ATCC 700603
   Proteus mirabilis ATCC 7002
   Pseudomonas aeruginosa ATCC 27853
   Salmonella enterica ATCC 14028
   Staphylococcus aureus ATCC 25923
   Staphylococcus aureus ATCC 29213
   Staphylococcus aureus ATCC 43300
   Staphylococcus aureus ATCC BAA-977
   Staphylococcus aureus ATCC BAA-1026
   Staphylococcus epidermidis ATCC 12228
   Staphylococcus saprophyticus ATCC 15305
   Streptococcus agalactiae ATCC 12386
   Streptococcus agalactiae ATCC 13813
   Streptococcus pyogenes ATCC 19615

2. On Wednesday, use the Blood agar subculture plate to perform the daily and weekly QC.
3. After QC is performed, discard previous week's subculture plates and store new plates in red QC plates bucket.

Weekly fastidious (Blood agar) organisms stock culture maintenance:

1. On Tuesday, subculture the following organisms from the previous weeks subculture plates for QC testing. Subculture organisms to Blood agar and incubate plates in CO_2 incubator at 35° overnight:

   Streptococcus pneumoniae ATCC 49619
   Streptococcus salivarius ATCC 13419
   Moraxella catarrhalis ATCC 25240

2. On Wednesday, use the Blood agar subculture plate to perform the daily and weekly QC.
3. After QC is performed, discard previous week's subculture plates and store new plates in "CO_2 plate" rack in CO_2 incubator.

Weekly fastidious (Chocolate agar) organisms stock culture maintenance:

1. On Tuesday, subculture the following organisms from the previous weeks subculture plates for QC testing. Subculture organisms to Chocolate agar and incubate plates in CO_2 incubator at 35° overnight.

   Aggregatibacter aphrophilus ATCC 7901
   Haemophilus influenzae ATCC 10211
   Haemophilus influenzae ATCC 49247
   Haemophilus influenzae ATCC 49766

2. On Wednesday, use the Chocolate agar subculture plate to perform the weekly QC.
3. Discard previous week's subculture plates and store new plates in "CO_2 plate rack".

As-required stock culture maintenance instructions

Vitek 2 GNI card organisms stock culture maintenance:

1. On the first day, subculture the following organisms from the Microbank vials for Vitek 2 GNI card QC testing. Subculture organisms to Blood agar and incubate plates in CO_2 incubator at 35° overnight:

   Enterobacter hormaechei ATCC 700323
   Stenotrophomonas maltophilia ATCC 17666
2. On the second day, subculture each organism to a new Blood agar plate. Incubate the plates in CO_2 incubator at 35° overnight.
3. On the third day, use the second Blood agar subculture plate to perform the Vitek 2 GNI QC testing.
4. After QC is performed, store plates in the red QC bucket until QC has been approved.

Vitek 2 GPI card organisms stock culture maintenance:

1. On the first day, subculture the following organisms from the Microbank vials for Vitek 2 GPI card QC testing. Subculture organisms to Blood agar and incubate plates in CO_2 incubator at 35° overnight:

   Enterococcus casseliflavus ATCC 700327
   Staphylococcus saprophyticus ATCC BAA-750
2. On the second day, subculture each organism to a new Blood agar plate. Incubate the plates in CO_2 incubator at 35° overnight.
3. On the third day, use the second Blood agar subculture plate to perform the Vitek 2 GPI QC testing.
4. After QC is performed, store plates in the red QC bucket until QC has been approved.

Vitek 2 NH card organism stock culture maintenance:

1. On the first day, subculture the following organism from the Microbank vial for Vitek 2 NH card QC testing. Subculture organism to Chocolate agar and incubate plate in CO_2 incubator at 35° overnight:

   Eikenella corrodens ATCC BAA-1152
2. On the second day, subculture organism to a new Chocolate agar plate. Incubate the plate in CO_2 incubator at 35° overnight.
3. On the third day, use the second Chocolate agar subculture plate to perform the Vitek 2 NH QC testing.
4. After QC is performed, store plate in the CO_2 incubator until QC has been approved.

Vitek 2 YST card organisms stock culture maintenance:

1. On the first day, subculture the following organisms from the Microbank vials for Vitek 2 YST card QC testing. Subculture organisms to Sabouraud agar and incubate plates at room temperature overnight:
   Candida albicans ATCC 14053
   Candida glabrata ATCC 2950
2. On the second day, subculture organisms to a new Sabouraud agar plate. Incubate the plates at room temperature overnight:
3. On the third day, use the second Sabouraud agar subculture plate to perform the Vitek 2 YST QC testing.
4. After QC is performed, store plates at room temperature until QC has been approved.

API 20 E organism stock culture maintenance:

1. On the first day, subculture the following organism from the Microbank vial for API 20 E QC testing. Subculture organism to Blood agar and incubate in CO_2 incubator at 35° overnight:
   Proteus mirabilis ATCC 35659
2. On the second day, subculture organism to a new Blood agar plate. Incubate the plate in CO_2 incubator at 35° overnight.
3. On the third day, use the second Blood agar subculture plate to perform the API 20 E QC testing.
4. After QC is performed, store plates in the red QC bucket until QC has been approved.

API NH organism stock culture maintenance:

1. On the first day, subculture the following organism from the Microbank vial for API NH QC testing. Subculture organism to Chocolate agar and incubate plate in CO_2 incubator at 35° overnight:
   Neisseria gonorrhoeae ATCC 31426
2. On the second day, subculture organism to a new Chocolate agar plate. Incubate the plate in CO_2 incubator at 35° overnight.
3. On the third day, use the second Chocolate agar subculture plate to perform the API NH QC testing.
4. After QC is performed, store plate in the CO_2 incubator until QC has been approved.

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.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven