INTERIM CULTURE METHOD FOR THE DUODENOSCOPE – DISTAL END AND INSTRUMENT CHANNEL

CDC Disclaimer: This protocol has not been validated. The protocol is still being developed and evaluated for the major duodenoscope types. This is an interim protocol and will be updated accordingly.

Purpose

This method is to culture bacteria from reprocessed duodenoscopes (after drying) specifically from the distal end and instrument channel. A laboratory will need to decide whether to process the samples with a Culture Method A - Presence/ Absence by Enrichment method or Culture Method B - Quantitative. The quantitative method also incorporates enriching the remainder of the sample to capture lower levels of contamination.

Sample Types: Instrument channel flush (50 ml)
Distal end and elevator mechanism, sampled by a channel-opening brush (submerged in 50 ml)

Materials and Reagents

- Vortex
- Incubator 35°C to 37°C
- Conical/ centrifugation tubes of various sizes tubes (50-cc, 1.5-cc)
- Sterile 0.01M phosphate buffered saline (PBS) with 0.02% Tween®-80 solution (PBST) (one example - Teknova, #P3875)
- Blood agar plates
- Selective agar (suggest MacConkey II agar plates for the detection of enteric pathogens)
- Tryptic soy broth (5 mL) (one example – Hardy Diagnostics, K89)
- Pipets and pipette tips

Culture Method A – Presence/ Absence by Enrichment

Note: Process irrigation water and PBST negative controls using the same protocol as the samples

1. Vortex the sample for 2 minutes in 10 second bursts
2. Aseptically, remove the channel-opening brush
3. Transfer the fluid samples (instrument channel flush, channel-opening brush fluid) to 50-cc conical tubes
4. Concentrate by centrifugation on a benchtop centrifuge equipped for high volume suspensions (range: 3,500 - 5,000 x *g* for 10 - 15 min).
5. Remove supernatant for a final volume of 1 mL without disrupting the pellet, or re-suspend the pellet to a final volume of 1 mL using PBST
6. Transfer the 1 mL sample to TSB (5 mL)
7. Incubate at 35°C to 37°C for 48 hrs
8. Check and record turbidity at 18 to 24 hrs (overnight) and 48 hrs
9. If the sample is turbid, streak broth for isolation onto blood agar and MacConkey II agar plates
10. Incubate at 35°C to 37°C; MacConkey II agar for 18- 24 hrs (overnight) and blood agar for 48 hrs
11. Observe plates for suspect colonies
12. Streak suspect colonies for isolation
13. Work up pure isolates for characterization of "low- concern" bacteria, which represent flora from skin and the environment, and species identification of "high-concern" bacteria.
    a. "Low-concern" bacteria include, but are not limited to, coagulase-negative staphylococci, micrococci, diptheroids, *Bacillus* spp. and other gram-positive rods
    b. "High-concern" bacteria include, but are not limited to, *Staphylococcus aureus*, *Enterococcus* spp., *Streptococcus* sp. viridians group, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp. and other enteric gram-negative bacilli.

Culture Method B - Quantitative

Note: Process the irrigation water and PBST negative controls using the same protocol as the samples

1. Vortex the sample for 2 minutes in 10 second bursts
2. Aseptically, remove the channel-opening brush

3. Transfer the fluid samples (instrument channel flush, channel-opening brush fluid) to 50-cc conical tubes
4. Concentrate by centrifugation on a benchtop centrifuge equipped for high volume suspensions (range: 3,500 - 5,000 x *g* for 10 - 15 min)
5. Remove supernatant without disrupting the pellet to a final volume of 1 mL. If needed, add PBST to a final volume of 1 mL and re-suspend.
6. Prepare a 1:10 dilution by adding 100 µl of sample to 900 µl of PBST
7. Vortex the sample for 10 sec
8. Pipet the following on to blood agar and MacConkey II agar plates in triplicate and spread evenly to allow for counting colonies
    a. 100 µl of the undiluted sample (final dilution 10^{-1})
    b. 100 µl 1:10 dilution (final dilution 10^{-2})
9. Add remainder of sample to TSB (5 mL) for enrichment in order to capture contamination below the detection limit
10. Incubate at 35°C to 37°C; MacConkey II agar for 18- 24 hrs (overnight), blood agar for 48 hrs, and TSB for 48 hrs
11. For agar plates: check and record growth at 18 to 24 hrs (overnight; MacConkey II and blood agar plates) and approximately 48 hrs (blood agar)
    a. Count and record number of colonies from plates
    b. Calculate CFU/sampled duodenoscope, accounting for the dilution of the sample
12. For TSB: check and record turbidity at 18 to 24 hrs (overnight) and approximately 48 hrs (two days)
    a. If the sample is turbid, streak broth for isolation on blood agar and MacConkey II agar plates
    b. Incubate at 35°C to 37°C; MacConkey agar for 18- 24 hrs (overnight) and blood agar for 48 hrs (two days)
    c. Observe plates for suspect colonies
13. Streak suspect colonies for isolation
14. Work up pure isolates for characterization of "low- concern" bacteria, which represent flora from skin and the environment, and species identification of "high-concern" bacteria.
    a. "Low-concern" bacteria include, but are not limited to, coagulase-negative staphylococci, micrococci, diptheroids, *Bacillus* spp. and other gram-positive rods
    b. "High-concern" bacteria include, but are not limited to, *Staphylococcus aureus*, *Enterococcus* spp., *Streptococcus* sp. viridians group, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp. and other enteric gram-negative bacilli.

Screening colonies for focused identification of "high-concern" bacteria

In this procedure, it is suggested that laboratories focus their efforts on species identification of "high-concern" bacteria to reduce workload. Characterize colonies with morphology consistent with those species using local clinical laboratory procedures. Facilities should consider using a rapid identification system (e.g. MALDI-TOF) for shortening turn-around times of results.

- **MacConkey agar:** Perform species identification of recovered GNR.
- **Blood Agar:** Characterize by hemolysis and perform preliminary tests (gram-stain, coagulase and other screening biochemicals) to rule out "low-concern" bacteria. Further species identification is required for "high-concern" bacteria.

Limitations

The sensitivity, specificity and limits on quantitation or detection are not established for all organisms with the specified processing methods.

This procedure focuses on the growth of "high-concern" organisms versus overall bioburden. To capture the overall bioburden, facilities may consider requiring lower temperatures of 30°C (±2) with an extended incubation time of 5-7 days for samples on additional blood agar plates.