PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Duodenoscope Culture Procedure

Department of Microbiology

Effective date: 03/23/2015

Last Revision: 03/23/2015

Last reviewed: 03/23/2015

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1.0 Purpose

This method is to culture bacteria from reprocessed duodenoscopes (after drying) specifically from the distal end and instrument channel.

2.0 Safety - Personal Protective Equipment

Performance of this procedure may expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

This procedure may expose you to:

- Enteric pathogens
- Bloodborne pathogens
- Airborne pathogens

To perform this procedure, you must use:

- Gloves – must be worn when handling samples.
- Laboratory Coat – must be worn when handling samples and cultures.
- Biological Safety Cabinet – must be used when processing samples.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

3.0 Samples Types

- Instrument channel flush (50 mL)

4.0 Materials

4.1 Equipment

- Vortex
- CO_2 incubator 35°C to 37°C

4.2 Consumables

- Conical centrifugation tubes (50-mL)
- Blood agar plates
- Sterile transfer pipettes

5.0 Procedure

1. Transfer 50 mL of the instrument channel flush fluid to a 50-mL conical tube.
2. Using the centrifuge in the AFB room, concentrate by centrifugation at maximum speed (3,060 x *g*) for 15 min.
3. Pour off the supernatant without disrupting the pellet. There should be about 0.5 to 1 mL of fluid remaining in the tube.
4. Re-suspend the pellet by using a vortex for 10 s.
5. Pipette half of the concentrated sample onto a blood agar plate, and the other half onto a another blood agar plate. Using an inoculation loop, spread the samples evenly on both plates by streaking a lawn to permit counting for colonies.
6. Incubate the plates at 35 to 37°C for 18-24 h.
7. Check for growth at 18-24 h and again at 48 h.
8. Count the total number of colonies on both plates.
9. Work up isolates for characterization of “low- concern” bacteria, which represent flora from skin and the environment, and identification of “high-concern” bacteria which represent potential organisms from the gastrointestinal tract.
    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.

6.0 Interpretation and Reporting of Results

1. Report the total number of colonies from both plates as CFU/scope.
2. Perform ID for any “low-concern” organisms. Report the identifications with no AST.
3. Perform ID and AST for any “high-concern” organisms. Specifically differentiate MRSA, VRE, ESBL, CRE, or any multi-drug-resistant organisms. Report the ID and AST results.

7.0 Limitations

1. The sensitivity, specificity and limits on quantitation or detection are not established for all organisms with the specified processing method.
2. This procedure focuses on the growth of “high-concern” organisms versus overall bioburden.

8.0 Validation Information

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.

9.0 References

1. Interim Culture Method for the Duodenoscope – Distal End and Instrument Channel. Centers for Disease Control and Prevention Website. http://www.cdc.gov/hai/pdfs/lab/interim-duodenoscope-culture-method.pdf Version 03/11/2015. Accessed 03/23/2015.

10.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 03/23/2015

Microbiology Supervisor Reviews: Jerry Claridge 03/24/2015

Revisions & Updates: