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

CDC Disclaimer: The sample and culture protocols were released as part of the larger CDC Interim Duodenoscope Surveillance Protocol as a guide for facilities considering cultures of duodenoscopes to assess the adequacy of their duodenoscope reprocessing. We recommend reading the CDC Interim Duodenoscope Surveillance Protocol first. Due to the increasing concerns regarding antimicrobial resistant bacteria, we focused on protocols that capture and identify “high-concern” bacteria. Facilities may expand their surveillance program to include additional organisms.

This protocol has not been validated and is one approach to culturing duodenoscopes. The protocol is intended to provide facilities who are considering culturing with a starting point for a protocol that can be adapted for use. 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 ‘ready-to-use’, fully 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 provides options of either centrifugation or membrane filtration, as well as enriching a portion of the remaining sample to capture lower levels of contamination.

Additional points to consider (see the Frequently Asked Questions document for further details)

- This testing is not meant to assure sterility of the duodenoscope.
- These samples are not clinical or patient samples, but are environmental from a medical device.
- Samples should be processed by personnel with understanding of microbiological principles for culturing and identification of common environmental and clinical bacteria.
- Due to the effect of a disinfectant neutralizer on the yield of duodenoscope cultures being unknown, an internal process positive control has been included in the protocol as an option for facilities to consider.
Sample Types:
Collectively, there should be at least a total of five (5) samples for processing for one duodenoscope. However, if the facility chooses to combine the instrument channel flush and the distal end/ elevator mechanism sample, there would be a total of four (4) samples for processing. If multiple duodenoscopes are sampled at the same time and the same stock of irrigation water and PBST are used, there would be only one set of negative controls for that group of samples.

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Volume</th>
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<tbody>
<tr>
<td>1.</td>
<td>Instrument channel flush (irrigation water)</td>
<td>45 ml</td>
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<tr>
<td>2.</td>
<td>Internal process positive control – aliquot of instrument channel flush spiked with organism(s)</td>
<td>5 ml</td>
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<td>3.</td>
<td>Distal end/ elevator mechanism (brush submerged in PBST)</td>
<td>50 ml</td>
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<td>4.</td>
<td>Negative control – irrigation water stock used for instrument channel flush</td>
<td>50 ml</td>
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<tr>
<td>5.</td>
<td>Negative control – PBST stock used for the distal end/ elevator mechanism sample</td>
<td>50 ml</td>
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Materials and Reagents

- Vortex
- Incubator 35°C to 37°C
- Sterile forceps
- 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)
  - Buffer options include Teknova (#P3875) and Hardy (#U334)
  - PBST can also be made in the laboratory under aseptic conditions
- Blood agar plates
- Selective agar (suggest: MacConkey agar plates for the detection of enteric pathogens)
- Tryptic soy broth (5 mL)
- Pipets and pipette tips
- Positive control organism (e.g. Staphylococcus aureus, Escherichia coli)
- If the facility chooses to use membrane filtration:
  - Large sidearm filtering flask
  - Vacuum line (to secure a pressure differential 138 to 207 kPa)
  - Filtration units (examples: Fisher Scientific funnel base assembly with clamp catalog #13-645-088; Fisher Scientific 300 mL funnel catalog #13-645-089, Fritted 47 mm base catalog #13-645-091, #8 silicone stopper; Pall Corporation magnetic filter funnels catalog # 4247)
  - 47 mm 0.45 µm white gridded membrane filters (example: Fisher catalog #09-719-555, Fisher Scientific)
  - Sterile forceps
Culture Method A – Presence/ Absence by Enrichment

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

1. Sterile brush used to sample the distal end: Vortex the sample for 2 minutes in 10 second bursts and aseptically remove the brush using sterile forceps from the PBST solution

2. Transfer the fluid samples (instrument channel flush, sterile brush fluid) to 50-cc conical tubes

3. Consider including an internal process positive control, which may provide insight on whether the culture protocol was conducted appropriately and if remaining residual disinfectant, if any, may have had an impact on organism viability and detection.
   a. Aliquot 5 ml of the instrument channel flush sample to a sterile conical tube. Reserve the remaining 45 mL for further processing as described below (see Step 4).
   b. Inoculate the 5 mL instrument channel flush sample with a gram-positive (e.g. *Staphylococcus aureus*) and/or a gram-negative (e.g. *Escherichia coli*) at a low inoculum (e.g. 100 colony forming unit (CFU))
   c. Process the internal process positive control sample as described in the following steps. Take precautions to not cross-contaminate the internal process positive control with the duodenoscope samples.

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 aspirate the supernatant and 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 h

8. Check and record turbidity at 18 to 24 h (overnight) and 48 h

9. If the sample is turbid, streak broth for isolation onto blood agar and MacConkey agar plates

10. Incubate at 35°C to 37°C; MacConkey agar for 18- 24 h (overnight) and blood agar for 48 h

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, diphteroids, *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. Sterile brush used to sample the distal end: Vortex the sample for 2 minutes in 10 second bursts and aseptically, remove the brush using sterile forceps from the PBST solution

2. Transfer the fluid samples (instrument channel flush, sterile brush fluid) to 50-cc conical tubes

3. Consider including an internal process positive control, which may provide insight on whether the culture protocol was conducted appropriately and if remaining residual disinfectant, if any, may have had an impact on organism viability and detection.
   a. Aliquot 5 ml of the instrument channel flush sample to a sterile conical tube. Reserve the remaining 45 mL for further processing as described below (see Step 4).
   b. Inoculate the 5 mL instrument channel flush sample with a gram-positive (e.g. *Staphylococcus aureus*) and/or a gram-negative (e.g. *Escherichia coli*) at a low inoculum (e.g. 100 CFU)
   c. Process the control sample as described in the following steps for the chosen method. Take precautions to not cross-contaminate the positive control with the duodenoscope samples.

4. Choose either I. Centrifugation or II. Membrane Filtration to concentrate the sample:

   **I. Centrifugation**
   1. Concentrate by centrifugation on a benchtop centrifuge equipped for high volume suspensions (range: 3,500 - 5,000 x g for 10 - 15 min)
   2. 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
   3. Prepare a 1:10 dilution by adding 100 µl of sample to 900 µl of PBST
   4. Vortex the sample for 10 sec
   5. Pipet the following on to blood agar and MacConkey agar plates in triplicate and spread evenly to allow for counting colonies
      i. 100 µl of the undiluted sample (final dilution 10⁻¹)
6. Continue with Step 5 in II. Membrane Filtration

II. Membrane Filtration

1. Set up membrane filtration equipment in a laboratory (e.g. sidearm filtering flask, vacuum, filter housings, gridded filters, sterile forceps, etc.)

2. The total volume needed to assay samples in duplicate on both blood and MacConkey agar plates is 40 mL at a minimum. Consider other volumes or dilutions depending on observed counts by the facility.
   a. Blood agar: 2 – 10 mL
   b. MacConkey agar: 2 – 10 mL

3. Filter the samples, making sure to rinse the filter housing liberally with a sterile buffered solution after each sample.

   Note: The minimum volume appropriate for membrane filtration is 10 mL. Thus, if filtering 1 mL samples, add at least 9 ml of a sterile buffered solution to the filter housing with the filter valve closed. Then, add the 1 mL sample to the sterile buffered solution and open the valve to allow for filtration.

4. Place the gridded filter using sterile forceps, grid side up, on the agar plate; taking care to place the filter completely flat and removing any air bubbles or creases in the filter.

5. Add up to 0.5 ml of the remaining sample to TSB (5 mL) for enrichment in order to capture contamination below the detection limit.

6. Incubate at 35°C to 37°C; MacConkey agar for 18-24 h (overnight), blood agar for 48 h, and TSB for 48 h.

7. For agar plates: check and record growth at 18 to 24 h (overnight; MacConkey and blood agar plates) and 48 h (blood agar).
   a. Count and record number of colonies from plates.
   b. Calculate CFU/mL from the blood agar plates and account for the volume of the sample filtered to determine the total CFU/sampled duodenoscope (50 mL sample).

8. For TSB: check and record turbidity at 18 to 24 h (overnight) and approximately 48 h (two days).
   a. If the sample is turbid, streak broth for isolation on blood agar and MacConkey agar plates.
   b. Incubate at 35°C to 37°C; MacConkey agar for 18-24 h (overnight) and blood agar for 48 h (two days).
c. Observe plates for suspect colonies

9. Streak suspect colonies for isolation
10. 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.

**Interpretation of post-reprocessing cultures and remedial actions**

For a complete interpretation of post-reprocessed cultures and remedial actions, please refer to the [CDC Interim Duodenoscope Surveillance Protocol](#) flow diagrams posted at the bottom of the webpage and/or the text related to (1) ‘testing after 60 ERCP procedures or once a month’ or (2) ‘testing after every duodenoscope reprocessing’. Briefly, assess the colony forming units (CFU) per duodenoscope and the following action levels:

- If <10 CFU of low-concern organisms per duodenoscope, reprocess and return to circulation.
- If >10 CFU of low-concerning organisms per duodenoscope, review facility-specific acceptable levels, reprocess and culture again if not below acceptable levels.
- If any number of high-concern organisms are identified, reprocess and culture again. Do not return to circulation until the cultures are negative or below the acceptable levels of low concern organisms.
If cultures are repeatedly positive for any high-concern organism or >10 CFU per duodenoscope for low-concern organisms, facilities should consider re-evaluating their culture technique and/or send the duodenoscope to the manufacturer.

**Troubleshooting abnormal negative and internal process positive control results:**

**Negative controls - Irrigation water and PBST**

- If there is bacterial growth in the negative controls, the duodenoscope samples have been compromised due to contamination and interpretation of the results will be questionable.
- The facility will need to troubleshoot the cause of contamination by evaluating personnel aseptic techniques, sampling area cleanliness, and the sterility and quality of the processing materials, stock solutions, and media.
- Consider using unopened and sterile materials to sample the duodenoscope again.

**Internal process positive control – Instrument channel flush aliquot (5 ml) spiked with Escherichia coli and/or Staphylococcus aureus**

- The facility should determine if the cultures used for the positive controls were viable and added at the correct concentration or dilution.
- If there is no bacterial growth (e.g. turbidity) in the enrichment culture using the Qualitative Method or if the CFUs are outside a factor of 2 from the inoculum control using the Quantitative Method (e.g., 30 CFUs isolated from the internal process positive control, and 65 CFUs isolated from the inoculum control), the duodenoscope samples may have been compromised due to improper conditions (e.g. incorrect or expired media, incorrect incubation time or temperature, concentration of HLD residual). Interpretation of the results will be questionable.
- Consider sampling the duodenoscope again after the improper conditions have been identified and corrected.

**Limitations**

The protocols are not yet validated, i.e. the sensitivity, specificity and limits in quantitation or detection are not established for all organisms.

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.