DATE: March 23, 2015

TO: Hospitals, Diagnostic and Treatment Facilities, Local Health Departments, NYSDOH Regional Epidemiologists, NYSDOH Wadsworth Center

FROM: NYSDOH Bureau of Healthcare Associated Infections

Health Advisory:
Interim Protocol for Healthcare Facilities Regarding Surveillance for Bacterial Contamination of Duodenoscopes after Reprocessing

Please distribute immediately to: Hospital Epidemiologists, Infection Preventionists, Laboratory Directors, Infectious Diseases Physicians, Gastroenterologists, Gastrointestinal Surgeons, Endoscopy Unit, Central Sterile Processing Unit, Medical Director, Nursing Directors, Risk Managers, and Biomedical Engineers

Recent reports have identified carbapenem-resistant Enterobacteriaceae (CRE) transmission associated with persistently contaminated duodenoscopes for which no breaches in reprocessing were identified. Refer to NYSDOH Health Advisory Design of Endoscopic Retrograde Cholangiopancreatography (ERCP) Duodenoscopes May Impede Effective Cleaning: FDA Safety Communication that was issued on February 20, 2015: https://commerce.health.state.ny.us/hpn/ctrldocs/alrtview/postings/Notification 18148.pdf

The Centers for Disease Control and Prevention (CDC) has issued an interim protocol for use of surveillance cultures to assess reprocessing of duodenoscopes. While these measures apply primarily to duodenoscopes, they can also be implemented for other flexible endoscopes that have an elevator mechanism (e.g., those used to perform endoscopic ultrasound). The interim protocol for duodenoscope surveillance is intended to supplement and not replace or modify manufacturer recommended reprocessing procedures.

NYSDOH recommends that all facilities using duodenoscopes carefully consider implementing this protocol, and NYSDOH strongly encourages facilities located in areas of high CRE incidence or prevalence to implement this protocol. Should an infection or outbreak associated with duodenoscope use be recognized, regardless of the organism, NYSDOH will recommend that surveillance cultures be instituted or performed at increased frequency. Such infections and outbreaks should also be reported to the local health department and to the NYSDOH Bureau of Healthcare Associated Infections, Healthcare Epidemiology and Infection Control (HEIC) Program.

CRE has emerged as a serious public health threat in New York State. While some hospitals have never reported a case, downstate hospitals, especially in the New York City area, carry a very high burden of CRE. Refer to Hospital-Acquired Infections New York State 2013 issued in the fall of 2014:

Related information: On March 12, 2015, the U.S. Food and Drug Administration announced new actions to enhance the safety of reusable medical devices and address the possible spread of infectious agents between uses. The new recommendations are outlined in a final industry guidance aimed at helping device manufacturers develop safer reusable devices, especially those devices that pose a greater risk of infection.
http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm437804.htm

Please contact the NYSDOH, Bureau of Healthcare Associated Infections, Healthcare Epidemiology and Infection Control (HEIC) Program at your Regional Office or at the Central Office (Albany) with any questions or concerns regarding this advisory:

Western Regional Office (716) 847-4503
Central New York Regional Office (315) 477-8166
Metropolitan Area Regional Office (914) 654-7149
Capital District Regional Office (518) 474-1142
Central Office (Albany) (518) 474-1142
Interim Protocol for Healthcare Facilities Regarding Surveillance for Bacterial Contamination of Duodenoscopes after Reprocessing

Outbreaks of bacterial infection associated with endoscopes are often attributed to improperly reprocessed endoscopes. However, recent reports have identified carbapenem-resistant Enterobacteriaceae (CRE) transmission associated with persistently contaminated duodenoscopes for which no breaches in reprocessing were identified [1].

There is currently very limited information to guide the use of surveillance cultures to assess endoscope reprocessing outside of recognized outbreak settings. Surveillance cultures are not a replacement for appropriate training and oversight of endoscope reprocessing practices. Before initiating surveillance cultures, facilities considering their use should involve key facility staff, including the clinical laboratory director, clinical staff, infection prevention staff, hospital epidemiologists, and risk management staff to develop a plan for implementation, and response (e.g., patient notification) to surveillance culture results.

The following considerations are intended for facilities that perform procedures using duodenoscopes to assess the adequacy of reprocessing. While these measures apply primarily for duodenoscopes, they can also be implemented for other flexible endoscopes that have an elevator mechanism (e.g., used to perform endoscopic ultrasound). This document is intended to supplement and not replace or modify manufacturer recommended reprocessing procedures. This is an interim protocol and measures outlined below may change as new information becomes available.

- **Duodenoscope Reprocessing**: Facilities should review all steps in duodenoscope reprocessing several times a year (e.g., quarterly) and ensure strict adherence to the manufacturer’s instructions, paying particular attention to the following:
  - **Inspection and manual cleaning**: Ensure that the elevator mechanism located at the distal tip of the duodenoscope is thoroughly cleaned and free of all visible debris. The visible inspection is to be done with the elevator in the “open/raised” position as well as with the elevator in the “closed/lowered” position to ensure there is no visible debris above or below the elevator mechanism. Consideration should be given to use of a magnifying glass (e.g., 10x) to improve detection of residual debris around the elevator mechanism.
  - **Drying**: Ensure that the channels of the duodenoscope and elevator mechanism are thoroughly dried prior to storage. This should include an alcohol flush followed by forced air drying if these procedures are compatible with the duodenoscope per the manufacturer’s instructions. If channels and the elevator mechanism are not completely dry, bacterial growth can occur, forming a biofilm that is difficult to remove and could result in persistent contamination.

- **Use of Duodenoscope Culturing**
  - **Surveillance**: Although routine culturing of endoscopes is not part of current U.S. guidelines, recent outbreaks associated with duodenoscopes have led some facilities to consider regular monitoring to assess the adequacy of duodenoscope reprocessing (see algorithm).
    - The optimal frequency of surveillance cultures has not been established. International guidelines have recommended intervals ranging from every 4 weeks to annually (2, 3). A facility choosing to perform surveillance cultures can consider performing post-reprocessing cultures periodically, e.g., monthly or after every 60 procedures for each duodenoscope. Some facilities could choose to perform duodenoscope cultures weekly (e.g., after procedures on Friday to allow cultures to incubate over the weekend). Alternatively, facilities can choose to perform cultures, after reprocessing, following each use.
    - Cultures should be obtained after the duodenoscope has been reprocessed (after drying) and should include at least the instrument channel and the distal end of the duodenoscope (i.e., elevator mechanism and elevator recess for duodenoscopes with sealed elevator wire channel; and elevator mechanism, elevator recess, and elevator channel for duodenoscopes with unsealed elevator wire channels). An interim sampling protocol developed by CDC that represents one approach to culturing of duodenoscopes is available at the following link (Interim Duodenoscope Sampling Method and Interim Duodenoscope Culture Method).
      - Facilities may choose other sampling methods (e.g., flush-brush-flush method), or choose to sample additional channels beyond those specified in this approach. The sensitivity of the interim protocol has not been determined. A negative culture does not completely exclude the possibility of a contaminated duodenoscope. However, positive culture results should lead to some action as described below.
Post-reprocessing cultures of duodenoscopes should be assessed for two types of microbial growth: high- and low-concern organisms. If successfully disinfected, culturing should not detect any high-concern organisms (i.e., organisms more often associated with disease), such as Gram-negative bacteria (e.g., *Escherichia coli*, *Klebsiella pneumoniae* or other Enterobacteriaceae, as well as *Pseudomonas aeruginosa*), *Staphylococcus aureus*, and *Enterococcus*. Small numbers of low-concern organisms (i.e., organism less often associated with disease and potentially a result of contamination of cultures during collection) might occasionally be detected (e.g., coagulase-negative staphylococci excluding *Staphylococcus lugdunensis*, *Bacillus* species, diphtheroids). The levels of these low-concern contaminants on a duodenoscope can vary depending on the reprocessing, handling, and culturing practices in a facility; levels of such organisms detectable after reprocessing will therefore vary. Facilities can monitor the levels of these bacteria within the first month of surveillance testing to develop an expected baseline for those organisms. Typically, fewer than 10 colony forming unit (CFU) of low-concern microbes does not require intervention; interpretation of culture results with ≥10 CFU of low-concern microbes should be considered in the context of typical culture results at the facility. Any quantity of high-concern organism (i.e., one colony or greater) warrants further remedial actions as described below. This is consistent with previous recommendations (2, 4).

- Holding duodenoscopes out of use while surveillance culture results are pending could be considered, especially if performing surveillance cultures after each use. Any duodenoscope found to be contaminated should not be returned to use until steps outlined in remedial actions section (below) are addressed.
- Facilities should ensure that each endoscopic procedure is appropriately documented with regard to the specific endoscope used in order to allow identification of exposed patients should microbial growth be detected as described above. Furthermore, results of post-reprocessing duodenoscope cultures should be logged and tracked for each duodenoscope.
- Non-culture methods (e.g., adenosine triphosphate (ATP) bioluminescence assays) have been used to assess duodenoscope reprocessing by detecting residual organic material after cleaning. While individual facilities might choose to use such non-culture assays, more work is needed to interpret their results since non-culture methods lack consistent correlation to bacterial concentrations. They might, however, provide insight regarding the quality of duodenoscope reprocessing if systematically validated (5, 6).
  
  **During outbreaks**
  - Surveillance cultures have been used during outbreaks to identify contaminated duodenoscopes and to ensure that ongoing contamination is not occurring
  - Until the limits of detection are defined, negative surveillance cultures alone should not be used to rule out duodenoscopes as a source of cross-transmission.
  - Following documented transmission of bacteria via a duodenoscope, facilities should consider performing a series (e.g., 3 to 5) of duodenoscope surveillance cultures after reprocessing to ensure that the interventions employed to address the issue have eliminated contamination and are preventing further contamination that could lead to transmission.
  - An interim sampling protocol developed by CDC is available at the following link ([Interim Duodenoscope Sampling Method](#)) and ([Interim Duodenoscope Culture Method](#)).

**Remedial Actions:** Any duodenoscope found to be contaminated with any high-concern organisms or unacceptable CFU of low-concern organisms should be reprocessed again with repeat post-reprocessing cultures obtained. The duodenoscope should not be used again until it has been demonstrated to be free of high-concern organisms and has an acceptable level of low-concern organisms. Positive cultures should prompt a procedure review to ensure adherence to the manufacturer’s reprocessing instructions and to ensure cultures are being performed correctly. If a reprocessing breach is identified, appropriate facility personnel (e.g., infection prevention staff) should be notified and corrective actions should be immediately implemented. Refer to the manufacturer’s instructions for evaluating the duodenoscope for defects when bacteria are persistently recovered by duodenoscope cultures (including repeated cultures positive for low-concern organisms). In this situation, the facility can consider having the duodenoscope evaluated by the manufacturer. In addition, when unsuccessful reprocessing is suspected based on surveillance cultures, it might be helpful to review positive cultures among affected patients to determine whether other clusters of relevant pathogens could have been transmitted.
**Patient Information and Notification:** Patients undergoing procedures using duodenoscopes should be informed during the consenting process that there is a risk of patient-to-patient bacterial transmission associated with the procedure, including uncommon transmission of a multidrug-resistant organism. Facilities should document the specific duodenoscope used for each patient to facilitate identification of the exposed patients if needed. If high-concern organisms are recovered from a reprocessed duodenoscope (as described above), the decision to notify patients of their potential exposure should be made in consultation with key facility staff, including involved healthcare providers, infection prevention staff, hospital epidemiologists, and risk management. In instances where a multidrug-resistant organism (e.g., CRE) is cultured from a reprocessed duodenoscope, screening of exposed patients for the organism should be considered (a laboratory protocol for rectal CRE screening is available in the CDC CRE toolkit: [http://www.cdc.gov/HAI/pdfs/labSettings/Klebsiella_or_Ecoll.pdf](http://www.cdc.gov/HAI/pdfs/labSettings/Klebsiella_or_Ecoll.pdf)). This allows for appropriate infection control precautions to be implemented during future admissions to a healthcare facility for any exposed patient with positive screening cultures for the multidrug-resistant organism. Detailed information on patient notifications is available at: [http://www.cdc.gov/infectioncontrol/pntoolkit/index.html](http://www.cdc.gov/infectioncontrol/pntoolkit/index.html).

**Staff Training and Competency:** Ensure personnel performing reprocessing of duodenoscopes have received appropriate training with competency verification for reprocessing procedures. Competencies should be assessed at initiation of employee duties and at least annually and anytime a breach is identified or when a new technique or equipment is introduced. Competency verification should include direct observation in addition to other assessments per facility policy (e.g., written tests). Personnel responsible for reprocessing endoscopes are encouraged to seek certification in flexible endoscope reprocessing.

5. Alfa MJ, Fatima I, Olson N. The adenosine triphosphate test is a rapid and reliable audit tool to assess manual cleaning adequacy of flexible endoscope channels. Am J Infect Control 2013;41:294-53
Testing duodenoscope after 60 ERCP procedures or once a month

Test duodenoscope and consider holding the instrument until culture results available. Culture method options: (A) Presence/Absence by Enrichment or (B) Quantitative

Negative
Reprocess again to remove PBST and return to circulation

Positive

Low-concern organisms
Examples: coagulase-negative staphylococci, micrococci, diphtheroids, Bacillus spp. and other gram-positive rods

Culture Method: Enrichment
1. Reprocess and culture again
2. Do not return to circulation until cultures are negative or are below acceptable levels of low-concern organisms †

OR

Culture Method: Quantitative
1. Quantify colonies, if <10 CFU/duodenoscope †, reprocess to remove PBST and return to circulation
2. If not <10CFU/duodenoscope, review facility-specific acceptable levels †, reprocess and culture again if not below acceptable levels
3. Do not return to circulation until cultures are negative or are below acceptable levels of low-concern organisms †

Any high-concern organisms
Examples: Staphylococcus aureus, Enterococcus spp., Streptococcus sp. viridans group, Pseudomonas aeruginosa, Klebsiella spp., Salmonella spp., Shigella spp. and other enteric gram-negative bacilli

1. Reprocess and culture again
2. Do not return to circulation until cultures are negative or are below acceptable levels of low concern organisms †
3. Consider notification of patients exposed to duodenoscope since last negative cultures

If cultures are repeatedly positive (3 times or more) for either any high-concern organism or >10 CFU/duodenoscope of low-concern organisms, facilities should consider re-evaluating their culture technique and/or sending the duodenoscope to the manufacturer for evaluation

† The levels of low-concern organisms on a duodenoscope may vary depending on the reprocessing, handling, and culturing practices in a facility. Therefore, the acceptable level of these organisms can vary. Facilities can monitor the levels of low-concern organisms during the first month of surveillance testing to develop an appropriate baseline for those organisms. Typically, fewer than 10 CFU of these microbes does not require intervention; interpretation of culture results with >10 CFU of non-pathogenic microbes should be considered in the context of expected culture results at the facility.

Definitions
Negative – A liquid enriched culture is not turbid
Positive – A liquid enriched culture is turbid
CFU – colony forming units
PBST – Phosphate buffered saline with Tween®-80 solution
**Testing after every duodenoscope reprocessing**

Test duodenoscope and hold the instrument until culture results available. Culture method options:
(A) Presence/Absence by Enrichment or (B) Quantitative

- **Positive**
  - Choose not to identify organism
  - Reprocess again and re-culture

- **Negative**
  - Reprocess again to remove PBST and return to circulation

- **Choose to identify organism**

- **Low-concern organisms**
  - Examples: coagulase-negative staphylococci, micrococci, diphtheroids, *Bacillus* spp. and other gram-positive rods

  - **Culture Method: Enrichment**
    1. Reprocess and culture again
    2. Do not return to circulation until cultures are negative or are below acceptable levels of low-concern organisms†

  - **Culture Method: Quantitative**
    1. Quantify colonies, if <10 CFU/duodenoscope†, reprocess to remove PBST and return to circulation
    2. If ≥10 CFU/duodenoscope, review facility-specific acceptable levels†, reprocess and culture again if not below acceptable levels
    3. Do not return to circulation until cultures are negative or are below acceptable levels of low-concern organisms†

- **Any high-concern organisms**
  - Examples: *Staphylococcus aureus*, *Enterococcus* spp., *Streptococcus* sp. viridians group, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp. and other enteric gram-negative bacilli

  1. Reprocess and culture again
  2. Do not return to circulation until cultures are negative or are below acceptable levels of low-concern organisms†

  - If cultures are repeatedly positive (3 times or more) for either any high-concern organism or >10 CFU/duodenoscope of low-concern organisms, facilities should consider re-evaluating their culture technique and/or sending the duodenoscope to the manufacturer for evaluation

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*This approach could be reserved specifically for patients known to be colonized or infected with or felt to be at high risk for multidrug-resistant organisms (e.g., carbapenem-resistant *Enterobacteriaceae*).

†The levels of low-concern organisms on a duodenoscope may vary depending on the reprocessing, handling, and culturing practices in a facility. Therefore, the acceptable level of those organisms present after reprocessing can vary. Facilities can monitor the levels of low-concern organisms during the first month of surveillance testing to develop an appropriate baseline for those organisms. Typically, fewer than 10 CFU of these microbes does not require intervention; interpretation of culture results with ≥ 10 CFU of low-concern organisms should be considered in the context of expected culture results at the facility.

**Definitions**

- **Negative** – A liquid enriched culture is not turbid
- **Positive** – A liquid enriched culture is turbid
- **CFU** – colony forming units
- **PBST** – Phosphate buffered saline with Tween®-80 solution
Interim Duodenoscopy Sampling Method

Interim Sampling 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; however, a version of this protocol has been used with Olympus, small intestinal videoscopes, models TJF-160VF and TJF-Q180V. This is an interim protocol and will be updated accordingly.

Purpose

This method is for use in the field to sample reprocessed duodenoscopes (after drying) for bacteria specifically located on the distal end; and for collecting samples from the instrument channel (via the instrument port to the distal end). Ideally, two personnel should perform this protocol, where one will hold the duodenoscope (facilitator) and the other person samples (sampler) accordingly. It is important to sample gently, while thoroughly, in order for optimal sampling and maintaining the integrity of the duodenoscope.

Materials and Reagents

- Sterile channel-opening brush, specific to the duodenoscope model manufacturer recommendations (one example - Olympus, #MH-507)
  - Note: Facility may choose to use the non-sterile disposable channel-opening brush but interpretations of positive cultures may be difficult
- Sterile 0.01M phosphate buffered saline (PBS) with 0.02% Tween®-80 solution (PBST) (one example - Teknova, #P3875)
- Sterile leak-proof specimen cups (120 mL) (one example - Fisher Scientific, #14-375-462)
- Sterile irrigation water (50 mL per duodenoscope)
- Sterile 60-cc syringes
- Additional materials: Parafilm®, Sterile alcohol pads, Hair coverings, Sterile gloves, Sterile diaper pads, Permanent marker, Face masks/shields, Sterile gowns, Tray with sterile liner, Labels

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http://www.cdc.gov/hai/settings/lab/lab-duodenoscope-sampling....
Definitions

Distal end – Includes the elevator mechanism (e.g. forceps elevator or elevator) and elevator recess for duodenoscopes with sealed elevator wire channel; and elevator mechanism, elevator recess, and elevator channel for duodenoscopes with unsealed elevator wire channel.

Lowered/ closed position - Notes the position of the elevator mechanism being parallel or within the elevator recess relative to the distal end of the duodenoscope, Figure 1a (#fig1).

 Raised/ open position - Notes the position of the elevator mechanism being perpendicular to the distal end of the duodenoscope, Figure 1b (#fig1).

Figure 1. Distal end, Model TJF-Q180V (Olympus) – Illustrating the orientation of forceps elevator in the (a) ‘lowered/ closed’ position and (b) ‘raised/ opened’ position (photos taken by CDC DHQP).

Method – Preparation of Materials

In Laboratory: Aseptically prepare specimen containers with PBST and sterile irrigation water if these reagents are not commercially available or already prepared

1. Prepare autoclavable channel-opening brushes: Gather and wrap one channel-opening cleaning brush for use on each duodenoscope in appropriately-sized autoclave pouches; autoclave using approved sterilization cycles available in healthcare laboratories (132°C for 4 minutes or 135°C at 3 minutes)
2. In a biological safety cabinet, aseptically prepare the following:
   1. Fill sterile leak-proof specimen containers with 50 mL PBST; label for brush samples with sample ID/ date
   2. Sterile irrigation water: aliquot 50 mL into sterile leak proof specimen containers; label for instrument channel flush with sample ID/ date
3. Repeat step (2) for the total number of duodenoscopes to be sampled
4. Save the remainder of stock PBST and irrigation water as negative controls

**In the area where the duodenoscope(s) will be sampled:**

1. Clean and disinfect the counter where sampling of the duodenoscope(s) will be performed with an EPA approved disinfectant for hard, non-porous surfaces observing manufacturer’s instructions on contact time and disinfection procedure
2. **Sampler and Facilitator:** Don sterile gowns, face masks/shields, hair coverings and gloves
3. Prepare the sampling materials by laying out the sterile diaper pad; placing respectively labeled sampling containers, pre-moistening PBST tubes in a rack, as well as other needed items (e.g. 60-cc syringes)
4. Gather sterile cleaning and channel-opening brushes for sampling of the duodenoscopes

**Method – Elevator mechanism and channel**

1. **Sampler and Facilitator:** Don sterile gloves
2. **Facilitator:** Sanitize the outer surface of the duodenoscope tip with a sterile alcohol pad, but use caution to **not wipe** the elevator mechanism and lens face at the distal end that will be sampled with the channel-opening brush (Figure 2 (#fig2)); allow to air dry prior to sampling. Place duodenoscope in tray with sterile liner until sampling
3. **Facilitator:** Obtain the channel-opening brush and open the pouch for sampler to access brush
4. **Facilitator:** Using the controller, set the **elevator mechanism in the lowered/closed position** (Figure 1a (#fig1)) and orient the distal end (relative to the **Sampler**) for optimal sampling
5. **Sampler:** Dip the channel-opening brush into the labeled PBST specimen container to pre-moisten the brush and press excess fluid from the brush inside the inner walls of the container
6. **Sampler:** Using the pre-moistened channel-opening brush, with twisting motion of the brush, sample the inside of the **elevator mechanism, recess, and channel in the lowered/closed position** (Figure 3a (#fig3))
7. **Facilitator:** Using the controller, set the elevator mechanism in the raised/open position (Figure 1b (#fig1)) and then orient the distal end (relative to the **Sampler**) for optimal sampling inside the mouth of the specimen container

**Figure 2.** Clean the outer surface of the duodenoscope tip with a sterile alcohol pad but
take care to not wipe the elevator mechanism and lens face (photo taken by CDC DHQP).

8. **Sampler**: Using the channel-opening brush, firmly brush under the **elevator mechanism in the raised/open position** (Figure 3b (#fig3)) and scrub the face of the lens (Figure 3c (#fig3)).

9. **Sampler**: Drop the channel-opening brush portion inside the mouth of the corresponding labeled (i.e. sample ID, date) specimen container.

10. **Facilitator**: Tighten the lid, and secure with Parafilm®

**Figure 3.** Sampling the elevator mechanism in the (a) ‘lowered/closed’ position, (b) ‘raised/opened’ position, and (c) sampling the elevator mechanism and lens face (photos taken by CDC DHQP).

**Method – Instrument Channel**

- **Sampler and Facilitator**: Replace and don sterile gloves if needed
- **Facilitator**: Obtain a sterile 60-cc syringe for the instrument channel sample, fill syringe with 50 mL sterile irrigation water from pre-filled and labeled specimen container, and hand to the **Sampler**
- **Facilitator and Sampler**: Coordinate how to hold the duodenoscope at the optimal angle for the **Sampler** to flush the instrument channel via the instrument port and the **Facilitator** to collect the sample in the specimen container
- **Sampler**: Flush the instrument channel with 50 mL of sterile irrigation water, specifically from the biopsy valve to the distal end to collect sample in sterile, labelled specimen container
- **Sampler**: Remove the 60-cc syringe and fill with air, then re-attach it to the instrument port and flush the air through the channel to flush out any remaining fluid into the sterile specimen container

• **Facilitator**: Tighten the lid, and secure with Parafilm®

### Storage and Shipping Considerations

- Samples should be placed at 4°C or on cold-paks for storage until further processing or shipping. Samples should not be stored beyond 24 hours after sampling.
- Save and send remainder of unused irrigation water and PBST for testing (negative controls).
- When packaging for overnight shipping:
  - Seal lids with Parafilm®;
  - Place each specimen container in its own ziplock bag;
  - Remainder of unused stock PBST and sterile irrigation water (negative control) in their own ziplock bags;
  - All bags with tubes may be placed in several large outer bags.

### Duodenoscope handling after sampling

After sampling is complete, we recommend reprocessing the duodenoscope according to manufacturer’s specifications while holding the scope until the microbial results are available.

### Limitations

Environmental sampling and processing methods are to be used for investigational or research purposes. The sampling efficiency of this method has not been established.
Interim Duodenoscopy Culture Method

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)

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Interim Duodenoscope Culture Method [PDF - 176 KB]

Interim Duodenoscope Surveillance Protocol

Interim Duodenoscope Sampling Method

**Sample Types:** Instrument channel flush (50 ml)
Distal end and elevator mechanism, sampled by a channel-opening brush (submerged in 50 ml)
• 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.
   1. “Low-concern” bacteria include, but are not limited to, coagulase-negative staphylococci, micrococci, diptheroids, *Bacillus* spp. and other gram-positive rods
   2. “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.
   1. “Low-concern” bacteria include, but are not limited to, coagulase-negative staphylococci, micrococci, diphtheroids, *Bacillus* spp. and other gram-positive rods
   2. “High-concern” bacteria include, but are not limited to, *Staphylococcus aureus*, *Enterococcus* spp., *Streptococcus* spp. 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.