

Duodenoscope Surveillance Sampling & Culturing

Reducing the Risks of Infection

Department of Health and Human Services Collaboration



U.S. FOOD & DRUG
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**AMERICAN
SOCIETY FOR
MICROBIOLOGY**

Duodenoscope Surveillance Sampling and Culturing Protocols

Developed by the FDA/CDC/ASM Working Group on Duodenoscope Culturing

The U.S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and American Society for Microbiology (ASM), together with duodenoscope manufacturers and other experts, developed the following standardized protocols for duodenoscope surveillance sampling and culturing. These protocols are an update to the Interim Duodenoscope Surveillance Protocol released by CDC in March 2015, and address the concerns regarding validation of duodenoscope culturing protocols raised in ASM's April 2015 Policy Statement on Culturing of Duodenoscopes. Duodenoscope culturing was one of the supplemental measures for duodenoscopes that emerged from an FDA-led expert panel meeting in May 2015 to address the infections associated with reprocessed duodenoscopes.

Duodenoscope surveillance culturing requires specific resources, training, and expertise, and not all health care facilities may be able to implement this type of testing. This document was developed to identify the types of resources and collaborative relationships required to conduct the testing, to standardize methods, and to establish expectations for culturing results. The recommendations in this document represent expert opinion based on current information as of February 2018.

Working Group Members:

FDA: Shani Haugen (corresponding author), Patricia Conville, Steve Elliott, Benjamin Fisher, Elizabeth Gonzalez, Victoria Hitchins, Sunny Park, Aftin Ross, Ribhi Shawar, Angel Soler-Garcia. CDC: Matt Arduino, Angela Coulliette-Salmond, Alex Kallen, Judith Noble-Wang. ASM Representatives: Kevin Alby (University of Pennsylvania), Eileen Burd (Emory University), Phyllis Della-Latta (Columbia University), Romney Humphries (UCLA), Nancy Miller (Boston University), Trish Simner (Johns Hopkins University), Punam Verma (Virginia Mason Medical Center), Kimberly Walker (ASM). Additional experts: Michelle Alfa (University of Manitoba), Nicole Goulding and Ben Tanner (Microchem Laboratory), Margret Oethinger (Providence Health and Services, Oregon), Alpa Patel and Christine Walton (Nelson Laboratories). Fujifilm: Keith Nelson. Olympus: Ed Arcscott, Shigeyoshi Baba, Takashi Kinebuchi, and Laura Storms. PENTAX Medical: Frank Canonica and Hudson Garrett Jr.

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Duodenoscope Surveillance Sampling and Culturing Overview and Protocols

Overview

- a. **Introduction:** Microbiological sampling and culturing of duodenoscopes involves sampling duodenoscope channels and the distal end of the duodenoscope, followed by culturing those samples with the goal of detecting bacterial contamination that may be present on the duodenoscope after reprocessing. Sampling and culturing endoscopes is a standard practice in some countries outside the United States to monitor the adequacy of endoscope reprocessing and to identify endoscopes with persistent contamination despite reprocessing. Current U.S. guidelines for endoscope reprocessing and infection control do not recommend endoscope surveillance sampling and culturing; however, some U.S. healthcare facilities have successfully implemented routine or periodic surveillance sampling and culturing of reprocessed duodenoscopes to provide an early notice of potential problems. This document provides recommendations for those healthcare facilities that choose to implement duodenoscope microbiological surveillance sampling and culturing.
 - i. **Purpose:** To provide protocols for surveillance sampling and culturing of reprocessed duodenoscopes intended as a quality control measure of the adequacy of reprocessing. These protocols are based on interim recommendations from the Centers for Disease Control and Prevention and guidelines from the Gastroenterology professional societies in Australia and Europe, reported experiences from U.S. hospitals, and additional testing conducted by manufacturers of duodenoscopes. The protocols are intended to minimize the workload for staff in healthcare facilities that choose to implement duodenoscope surveillance sampling and culturing while maximizing the potential for detecting viable microbes. The sampling fluid, sampling locations, and culture conditions and media were selected with those goals in mind. The primary focus of this protocol is for the detection of organisms of concern, some of which have been associated with infectious outbreaks. However, these protocols are not intended to be used during a suspected outbreak linked to inadequately reprocessed endoscopes, and results after following these protocols cannot be used to certify that an endoscope is sterile.
 - ii. **Scope:** Culturing information may be collected to monitor the facility-specific procedures for reprocessing duodenoscopes, and could be used to identify systematic errors in reprocessing or damaged endoscopes and equipment. The protocol is designed to identify most organisms of concern that could be present on a duodenoscope. The protocols are not intended to culture all microbes that could potentially contaminate a

flexible duodenoscope. Sampling and culturing protocols, including establishing microbial cutoff limits for low/moderate-concern organisms, should be established by each individual facility and may involve the following groups within a healthcare facility: Endoscope Reprocessing, Sterile Processing, Infection Prevention and Control, Risk Management, Clinical Microbiology or Laboratory Medicine, Gastroenterology/Gastrointestinal Surgery and Management. In this protocol, two to three specific locations on the duodenoscope will be sampled; sampling of additional channels may be performed based on the needs and resources of the healthcare facility (see Appendix 1). The protocols in this document are intended for surveillance sampling and culturing of duodenoscopes outside of outbreak investigations. The protocols were written specifically for duodenoscopes. If other types of endoscopes are tested, healthcare facilities should carefully consider the types of protocol modifications that would be required to sample from other types of flexible endoscopes (see Appendix 1). Among all flexible endoscopes, duodenoscopes were selected to be the focus of this protocol because of the reports of infections associated with this specific type of reprocessed endoscope. In the event of an outbreak of infection linked to an endoscope, a healthcare facility's sampling and culturing protocols may significantly differ from those described in this document (see section A5.1.1). Surveillance sampling and culturing, if performed, should not be a substitute for a comprehensive endoscope reprocessing program that includes but is not limited to complete adherence to professional guidelines and the manufacturer's reprocessing and maintenance recommendations.

- iii. **Organization:** This document is divided into three sections. The first section of the document provides an overview and introduction to the sampling and culturing protocols. The second section (Section A) outlines the materials and methods for duodenoscope sampling, to be conducted by appropriate personnel who are familiar with handling duodenoscopes (e.g. endoscopy or reprocessing staff). The third section (Section B) outlines four different methods for culturing samples from duodenoscopes, to be conducted by appropriate microbiology laboratory staff, and includes suggested initial limits for microbiological cut-offs based on expert opinion. Appendix 1 includes suggested volumes for endoscope channels of various sizes. Appendix 2 includes photographs of duodenoscope sampling to illustrate duodenoscope design features and sampling equipment.
- iv. **Protocol Adoption:** The protocols described in Sections A and B offer multiple options. Individual facilities may select the options most appropriate for their needs and resources, and reformat and re-word the

protocols to conform to the facility's institutional formatting for standard operating procedures. The methods themselves are not intended to be modified, except where options are specifically identified. For details regarding the brush specifications used during the sampling process, contact the duodenoscope manufacturer.

- v. **Sampling and Culturing Validation:** Three manufacturers of duodenoscopes in the U.S. have completed benchtop testing to validate the sampling and culturing protocols (data not shown). The studies were conducted by depositing small numbers of *Staphylococcus aureus* and *Escherichia coli* in the elevator recess, instrument channel, and elevator wire channel (where applicable) of their duodenoscopes. Sampling was conducted using the appropriate protocols described in this document and culturing was conducted using the filtration and plating approach. Adherence to the protocols recovered between 65% to 100% of microbes placed on the duodenoscope. The data suggest that the protocol can extract most, but not necessarily all, microbes on the device. Modification of the protocol, in any way, may negatively impact the microbial recovery from duodenoscopes. Since the surveillance sampling and culturing protocols are intended to monitor that the facility-specific procedures for reprocessing duodenoscopes render the devices properly reprocessed, and not that a particular duodenoscope is free of microbes, and because samples from duodenoscopes are not clinical specimens from patients and are not used for diagnostic purposes, it is not necessary for healthcare facilities to validate that they can successfully carry out the protocols and meet pre-determined acceptance criteria. The methods required for validation of the surveillance sampling and culturing protocols (e.g., intentional contamination of duodenoscopes with Gram-negative and Gram-positive bacteria) are not appropriate for duodenoscopes in healthcare facilities that will be used in patients. However, healthcare facilities may wish to assess staff proficiency in carrying out the protocols. Please see sections A4 and B4 of this protocol for discussion of proficiency assessments of sampling staff and culturing staff.

- vi. **Rapid ATP Testing:** Residual adenosine triphosphate (ATP) on cleaned reusable medical devices has been used as a marker of an inadequate cleaning process. Detection of ATP after cleaning of a medical device may represent residuals from patient secretions (as human cells and secretions have high ATP levels), as well as residuals from bacteria (bacteria have low ATP levels and high numbers of bacterial cells are required to elicit a positive ATP reading). Cleaning is expected to remove both patient-derived secretion and patient-derived bacteria. As such, ATP monitoring after cleaning is a measure of cleaning adequacy and does not

differentiate where the ATP originated from (i.e. from human secretions or bacteria). Furthermore, ATP tests cannot distinguish between high-concern and low/moderate-concern organisms. For these reasons, rapid ATP testing, while useful as a marker for the cleaning process, is not sufficiently sensitive to be used as a marker for the adequacy of the high level disinfection process. For healthcare facilities that choose to assess the quality of their cleaning and high level disinfection processes, ATP tests may be incorporated into a facility's procedures after the cleaning process, but ATP tests should not be used in lieu of endoscope sampling and culturing after high level disinfection. Testing cleaning adequacy after high level disinfection is also not appropriate because the intent of cleaning monitoring is to identify and correct inadequate cleaning processes prior to disinfection or sterilization.

- vii. **Regulatory Requirements:** Except in areas where endoscope surveillance sampling and culturing is specifically required or regulated by state or local authorities, use of this protocol is not currently required as part of a duodenoscope reprocessing program. Healthcare facilities that adopt endoscope surveillance sampling and culturing as a required policy should follow their own written procedures, including any necessary remediation activities outlined in their policy. Samples from endoscopes are not clinical specimens from patients, they are not used for diagnostic purposes, and they are not used to certify an endoscope as sterile. As such, sampling endoscopes for microbial culturing as a quality indicator of validated endoscope reprocessing procedures is not subject to CLIA oversight or FDA review.

b. References

- i. Interim Duodenoscope Surveillance Protocol: Interim Protocol for Healthcare Facilities Regarding Surveillance for Bacterial Contamination of Duodenoscopes after Reprocessing:
<https://www.cdc.gov/hai/organisms/cre/cre-duodenoscope-surveillance-protocol.html>
- ii. Gastroenterological Society of Australia 2010. Infection Control in Endoscopy – Microbiological testing of gastrointestinal and respiratory endoscopes and automated flexible endoscope reprocessors.
- iii. U. Beilenhoff., C.S. Neumann, J.F. Rey, H. Biering, R. Blim, V. Schmidt, and the ESGE Guidelines Committee 2007. ESGE-ESGENA guideline for quality assurance in reprocessing: Microbiological surveillance testing in endoscopy. *Endoscopy*. 39:175-181.

- iv. Supplemental Measures to Enhance Duodenoscope Reprocessing: FDA Safety Communication, accessed March 23, 2016.
- v. Guideline for processing flexible endoscopes. In: Guidelines for Perioperative Practice. Denver, CO: AORN, Inc; 2017:717-800.
- vi. ANSI/AAMI ST91:2015. Comprehensive guide to flexible and semi-rigid endoscope processing in health care facilities. Association of Advancement Medical Instrumentation, Arlington, VA.
- vii. CDC Patient Notification Toolkit:
<https://www.cdc.gov/injectionsafety/pntoolkit/index.html>, accessed August 12, 2016

c. Records

- i. All endoscope culturing records should be maintained by the healthcare facility's Infection Prevention and Control staff or other designated staff, for a duration of time determined by the healthcare facility.
- ii. Refer to Sections A7 and B7 for a summary of the information to document.

d. Responsibilities

- i. Duodenoscope surveillance sampling and culturing is a shared responsibility of multiple departments within a healthcare facility. Responsible departments may include, but are not limited to, Endoscope Reprocessing or Sterile Processing, Infection Prevention and Control, Risk Management, Clinical Microbiology or Laboratory Medicine, Gastroenterology/Gastrointestinal Surgery, and Management within a healthcare facility. When culturing is contracted to an Environmental/Reference laboratory outside of the healthcare facility, the healthcare facility remains responsible for reviewing and approving the culture procedures used by the contract laboratory.
- ii. Responsible departments should determine the following as they relate to endoscope surveillance sampling and culturing in their institution: the frequency of sampling and culturing (including the choice of periodic sampling and culturing based on a period of time or number of procedures, or the option to sample and culture after every use of the device), clinical use of duodenoscopes while awaiting culture results,

endoscopes to be sampled, additional endoscope channels to be sampled, endoscope handling after processing, how samples should be accessioned (received in the laboratory to track workflow), culture reporting format, identification of staff receiving culture results, training and proficiency assessment for sampling and culturing staff, duration of time to maintain endoscope culturing records, threshold limits for low/moderate-concern organisms, and frequency of review of this protocol.

- iii. Responsible departments share a joint responsibility in ensuring the healthcare facility has the resources to fulfill the endoscope surveillance sampling and culturing plan.
- iv. Responsible departments should jointly develop procedures to respond to endoscope culturing results, including when results exceed the pre-determined microbial limits. Examples of responses may include quarantine of endoscopes, additional reprocessing of the endoscope, retraining reprocessing staff on endoscope reprocessing protocols, conducting a risk/safety management response, and patient notification. See Section B6 for examples of microbial limits and actions.
- i. Infection Prevention and Control (or other designated management staff) should be responsible for timely review of culture reports and for documentation of results. They should also be responsible for ensuring appropriate remedial action is taken when culture results exceed the pre-determined microbial limits.

e. Definitions

- i. **ACTION:** Steps taken by a healthcare facility after growth of unacceptable microbial levels/organisms from an endoscope sample. See Section B6, Table 1.
- ii. **ALERT:** Steps taken by a healthcare facility after growth of elevated numbers (e.g., 11 – 100 CFU) of low/moderate-concern organisms that is below the Action level (e.g., > 100 CFU) for those organisms. Growth of low/moderate-concern organisms at the Alert level may warrant a review of reprocessing, sampling, and culturing protocols. See Section B6, Table 1.
- iii. **BIOPSY PORT:** The entrance to the instrument channel on the endoscope. The biopsy port is where accessory instruments (such as biopsy forceps or guidewires) are introduced into the endoscope.

- iv. **CHANNELS:** Duodenoscopes have multiple channels (long, narrow lumens) that have different functions during endoscopy. For example, the instrument channel allows insertion of accessory instruments into the endoscope for biopsy or therapeutic reasons, the air channel provides air for insufflation of the gastrointestinal tract, and the water channel provides water that may be used to wash the lens at the distal end of the duodenoscope.
- v. **CONTAMINATION:** A sample positive for any number of high-concern organisms or > 100 CFU of low/moderate-concern organisms. The presence of this type of contamination on a reprocessed endoscope may or may not lead to infection or colonization of patients by those organisms.
- vi. **CONTROL HANDLE:** The location of the endoscope that is handled by the physician during an endoscopy procedure. The control handle includes the buttons and knobs for control of the optics, movement of the distal portion of the endoscope, air insufflation, and lens washing.
- vii. **CULTURING PROTOCOL:** The series of steps applied to the liquid endoscope sample for the purpose of promoting growth and subsequent identification of high or low/moderate-concern organisms from that endoscope sample. See Section B of this protocol.
- viii. **DISTAL END:** The distal end includes the terminal end of the insertion tube that is inserted into the patient during the endoscopic procedure. This location of the duodenoscope also includes the elevator lever and elevator recess (Figure 1).
- ix. **ELEVATOR LEVER:** Also known as an elevator, the elevator lever is located at the distal end of the duodenoscope. It is a small piece, usually metal, that can pivot. When raised, the elevator lever changes the angles of accessory instruments exiting the instrument channel at the distal end, up to a nearly 90° angle.
- x. **ELEVATOR RECESS:** The recessed area surrounding the elevator lever. This area has numerous small crevices, making the elevator recess a particularly challenging area to clean (Figure 1).
- xi. **ELEVATOR WIRE CHANNEL:** The elevator wire channel is an extremely narrow channel within duodenoscopes that houses a thin wire that spans from the control handle of the duodenoscope (the portion of the duodenoscope in the physician's hands) to the elevator lever. This thin wire controls the movement of the elevator lever. In older models of

duodenoscopes, the elevator channel was exposed to patient bodily fluids and required reprocessing after each patient use. Some models of duodenoscopes have a closed (sealed) elevator wire channel, preventing access to the elevator wire channel.

- i. **ELEVATOR WIRE CHANNEL PORT:** This port is located on the control handle of duodenoscopes with open (unsealed) elevator wire channels, and allows for reprocessing and sampling of the elevator wire channel. This port is not present on closed (sealed) elevator wire channel duodenoscopes.
- ii. **ENDOSCOPE SAMPLE:** In this document, an endoscope sample is the liquid sample that was used to extract microbes from an endoscope and is used in subsequent culturing. An endoscope sample may also be referred to as “sample.”
- iii. **HIGH-CONCERN ORGANISM:** Organisms that are more often associated with disease. Examples of high-concern organisms include Gram-negative rods (e.g., *Escherichia coli*, *Klebsiella pneumoniae* or other *Enterobacteriaceae* as well as *Pseudomonas aeruginosa*), Gram-positive organisms including *Staphylococcus aureus*, Beta-hemolytic *Streptococcus*, *Enterococcus* species, and yeasts.
- iv. **HIGH LEVEL DISINFECTION:** A lethal process utilizing a sterilant under less than sterilizing conditions. The process kills all forms of microbial life except for large numbers of bacterial spores.
- v. **INSTRUMENT CHANNEL:** The channel spanning from the biopsy port to the distal end. This channel is used to insert instruments into the endoscope for use during endoscopy. The instrument channel forms a part of the suction channel, which extends from the distal end to the proximal end of the endoscope (the suction channel is not shown in the diagram in Figure 1).
- vi. **LOW/MODERATE-CONCERN ORGANISM:** Organisms that are less often associated with disease; their presence could result from environmental contamination during sample collection. Examples of low-concern organisms include many species of Gram-positive bacteria such as *Micrococcus*, coagulase-negative staphylococci (excluding *Staphylococcus lugdunensis*), as well as *Bacillus* and diphtheroids or other Gram-positive bacilli whose presence on a duodenoscope could be attributed to environmental contamination during sampling or culturing. Moderate-concern organisms consist of those commonly found in the oral cavity (e.g., saprophytic *Neisseria*, viridans group streptococci, and

Moraxella species).

- vii. LOWERED POSITION: The position for the elevator lever being parallel or within the elevator recess relative to the distal end of the duodenoscope.
- viii. MODIFIED ACTION: Action prompted as a consequence of the growth of low/moderate-concern organisms from an endoscope sample in liquid culture, including re-sampling and plating on blood agar. See Section B6.
- ix. RAISED POSITION: The position of the elevator lever such that it is perpendicular to the distal end of the duodenoscope.
- x. REPROCESSING: Validated processes used to render a medical device, which has been previously used or contaminated, fit for a subsequent single use. These processes are designed to remove soil and contaminants by cleaning and to inactivate microorganisms by disinfection or sterilization.
- xi. SAMPLING PROTOCOL: The series of steps conducted to extract microbes from the endoscope for the purpose of culturing that liquid extract. See Section A of this protocol.
- xii. STERILIZATION: A validated process used to render product free from viable microorganisms.

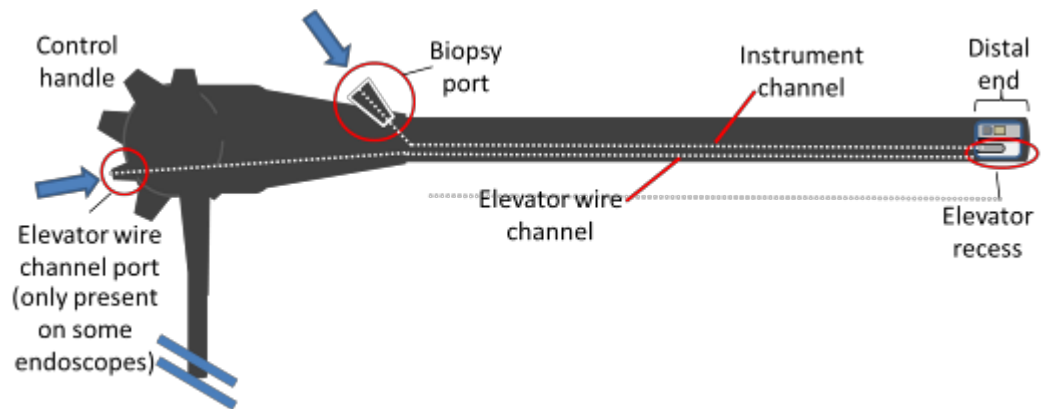
A Duodenoscope Surveillance Sampling Protocol

A1.0 Introduction

- A1.1 Purpose: To provide general recommendations for collecting samples from duodenoscopes for culturing.
- A1.2 Scope: The sampling protocol is limited to obtaining samples from the elevator recess and instrument channel, as well as the elevator wire channel (when accessible) of clinically used and reprocessed duodenoscopes for the purposes of surveillance. Some models of duodenoscopes have a closed (sealed) elevator wire channel, preventing access to the elevator wire channel. This protocol can be used to sample the elevator wire channel from duodenoscopes that have an open (or unsealed) elevator wire channel. Refer to Appendix 1 for information on sampling from other endoscope channels. The primary focus of the following protocols will be on organisms of concern that, some of which have been associated with infectious outbreaks due to contaminated duodenoscopes. The sampling method described below is not intended to be used during an investigation of infections associated with

reprocessed endoscopes. In that situation, a healthcare facility's sampling method may differ significantly from the protocol described below (e.g., sampling from all channels of the endoscope, channel/location-specific culturing, alternative sampling fluids, etc.). Guidance on sampling during outbreaks is not included in this document.

Figure 1: Duodenoscope diagram



A2.0 Safety

- A2.1 Collection of samples may generate aerosols. Wear appropriate personal protective equipment (PPE) when handling the endoscopes for sampling. See Section A5.2.1 for list of appropriate PPE.
- A2.2 Although endoscope samples are not clinical samples and the endoscope has been reprocessed, the endoscope has previously been in contact with a patient and appropriate precautions should be taken. All samples from endoscopes should be considered potentially biohazardous, and, therefore, the use of standard precautions and appropriate PPE for collecting, handling, and transporting these samples is recommended.
- A2.3 To facilitate aseptic sampling, an uncluttered countertop in a clean room or a clean surface, covered with an impervious sterile pad or drape should be used in conjunction with appropriate PPE including sterile gloves.
- A2.4 Adhere to the healthcare facility's procedures for biohazardous waste collection and disposal for single-use and reprocessing of reusable materials (e.g., gloves, syringes, brushes, flushing ports, etc.).
- A2.5 Depending on the transport method and conditions, healthcare facilities should consider the use of secondary barriers for endoscope samples to

minimize the risk of damage to sample containers and fluid leakage.

- A2.6 When endoscope samples are transported to an off-site laboratory, they must be labeled with biohazard designations and transported according to approved institutional protocols for biohazard sample transport by a certified individual and labeled appropriately (e.g., UN 3373).
- A2.7 Duodenoscopes themselves should not be transported off-site for the purposes of surveillance sampling and culturing. Transport can allow proliferation of microbes on the endoscope, and transport increases the potential for environmental contamination of the endoscope. Consider the need to transport the duodenoscope, such as when culture results are repeatedly positive or when damage is identified, necessitating return of the duodenoscope to the device manufacturer. Consult with the endoscope manufacturer regarding any special precautions that must be taken prior to shipping an incompletely reprocessed or contaminated duodenoscope to the manufacturer for service and/or repair.

A3.0 Responsibilities

- A3.1 Sampling should be conducted by staff who are competent with handling, using, or processing duodenoscopes (e.g., endoscope reprocessing technicians or endoscopy staff) and who have been trained on endoscope sampling and aseptic technique.
- A3.2 Appropriate management within the healthcare facility should ensure that sampling staff have received training on aseptic technique and endoscope sampling, and have demonstrated initial proficiency on endoscope sampling.
- A3.3 Sampling staff are responsible for sampling in accordance with the healthcare facility's sampling plan, documentation of sampling, arranging transport of samples to the microbiology laboratory for culturing, and handling of the endoscope after sampling.
- A3.4 Infection Prevention and Control staff (or other designated Management staff) are responsible for timely reviewing of endoscope culture reports, notifying appropriate management when results exceed acceptable limits, and ensuring any remedial action plans are immediately executed.

A4.0 Sampling Proficiency

- A4.1 Currently, there are no recommended external proficiency testing (EPT) programs or test methods that healthcare staff can use to demonstrate proficiency with endoscope sampling.

- A4.2 Samples from endoscopes are not clinical specimens from patients, they are not used for diagnostic purposes, and they are not used to certify an endoscope as sterile. As such, sampling endoscopes for microbial culturing as a quality indicator of endoscope reprocessing is not subject to CLIA oversight or FDA review.
- A4.3 Healthcare facilities that choose to implement endoscope sampling and culturing should conduct periodic audits of the sampling process to visually verify that staff are adhering to the facility's sampling protocol.

A5.0 Duodenoscope Sample Collection Protocols

A5.1 Overview: Two staff are required to conduct sampling from channels: one staff person (the sampler) maintains aseptic handling and conducts brushing steps, while the second staff person (the facilitator) opens packages and handles the unsampled portions of the endoscope. At a minimum, two samples should be collected and combined: an instrument channel sample (biopsy port to distal end) should be taken using a flush, brush, flush method, and an elevator recess sample should be taken by flushing and brushing of the elevator recess. For duodenoscopes with an open (unsealed) elevator wire channel, a third sample should be collected from the elevator wire channel by flushing that channel and combining the sample with the other two samples.

A5.1.1 The protocol in this document is intended for surveillance sampling of duodenoscopes, and not for outbreak investigations. In the event of an outbreak of infection linked to reprocessed endoscopes, a healthcare facility's sampling protocol may differ significantly from this protocol to maximize the sensitivity of the sampling (e.g., sampling from all channels of the endoscope, channel/location-specific culturing, alternative sampling fluids, etc.). Additional locations on the endoscope (e.g., air/water channel and suction channel) can be sampled based on the needs and resources at a facility (see Appendix 1 for further information).

A5.2 Materials

A5.2.1 Personal Protective Equipment (fresh PPE should be used with each endoscope sampled)

A5.2.1.1 Fluid-resistant sterile gown

A5.2.1.2 Fluid-resistant face mask and eye protection

- A5.2.1.3 Sterile gloves
- A5.2.1.4 Bouffant caps for hair
- A5.2.2 Surface disinfectant for counter top
- A5.2.3 Sterile fluid-resistant pad or drape
- A5.2.4 10x magnifying glass
- A5.2.5 Sterile collection container (minimum capacity of 100 mL)
- A5.2.6 (Optional) Step stool
- A5.2.7 Specimen labels and water-proof marker pens
- A5.2.8 Sterile Dey-Engley (DE) Broth or other appropriate neutralizer solution (45 mL)
- A5.2.9 Sampling fluid - Sterile non-bacteriostatic water, preferably sterile reverse osmosis or deionized water (45 mL)
- A5.2.10 Sterile alcohol wipe
- A5.2.11 Sterile swab that is capable of having the swab head removed
- A5.2.12 Sterile pipettes or sterile plastic transfer bulbs with markings for a 1 mL volume (2 pipettes or bulbs)
- A5.2.13 Sterile brush for elevator recess, meeting the following parameters:
 - A5.2.13.1 Provided sterile or capable of being steam sterilized onsite
 - A5.2.13.2 The brush should have a handle portion to facilitate ease of brushing the elevator recess
 - A5.2.13.3 Head of the bristled portion must be able to be aseptically removed from brush and transferred to sample container
 - A5.2.13.4 Bristle diameter: 2.3mm to 12mm - as specified by duodenoscope manufacturer

- A5.2.13.5 Bristle material: nylon or equivalent - as specified by the duodenoscope manufacturer
 - A5.2.13.6 Length of bristled portion: 9 to 44 mm
 - A5.2.13.7 Length of brush: 8 to 14 cm - as specified by duodenoscope manufacturer
 - A5.2.13.8 Brush tip feature: Plastic-tipped or equivalent as specified by the duodenoscope manufacturer to minimize gouging internal duodenoscope surfaces
- A5.2.14 Sterile scissors or sterile wire cutters
- A5.2.15 Sterile 30 mL syringes (2 syringes)
- A5.2.16 Sterile brush for instrument channel, meeting the following parameters:
- A5.2.16.1 Head of the bristled portion must be able to be aseptically removed from brush and transferred to collection container
 - A5.2.16.2 Bristle diameter: 4 to 6 mm - as specified by duodenoscope manufacturer
 - A5.2.16.3 Bristle material: nylon or equivalent – as specified by duodenoscope manufacturer
 - A5.2.16.4 Length of bristled portion: minimum of 8 mm
 - A5.2.16.5 Length of brush: minimum of 210 cm – as specified by duodenoscope manufacturer
 - A5.2.16.6 Brush tip feature: Plastic-tipped or equivalent as specified by duodenoscope manufacturer to minimize gouging internal duodenoscope surfaces
- A5.2.17 A countertop or table long enough to lay the scope out for inspection
- A5.2.18 When sampling from open elevator wire duodenoscopes, the following materials are necessary:
- A5.2.18.1 Sterile 5 mL luer lock syringe

A5.2.18.2 Elevator wire channel washing/flushing/cleaning tube/adapter, sterilized

A5.2.18.3 An additional 3 mL of sterile water for sampling

A5.2.19 Ice, ice pack, or refrigerator (2 - 8°C)

A5.2.20 If using an off-site laboratory for culturing, an appropriately validated shipping/transport container system for sample delivery

A5.3 Methods

A5.3.1 Sampling preparation

A5.3.1.1 Label the sterile sample container with appropriate identifying information (e.g., duodenoscope device number, channel/sites sampled, date, time, and identification of sampler).

A5.3.1.2 Facilitator and Sampler: Don fluid-resistant face mask and eye protection. Disinfect counter with appropriate surface disinfectant starting from back of counter working towards front. Perform hand hygiene. Don remaining PPE (bouffant cap, gown, gloves).

A5.3.1.3 Facilitator: Place sterile pad or drape on counter. Retrieve duodenoscope and place on sterile pad or drape, taking care to avoid contact with the elevator recess.

A5.3.1.4 Sampler: Before sampling the duodenoscope, perform a visual inspection of its distal end for any debris or other concerns using 10x magnification.

A5.3.1.4.1 If visible debris is present, continue with sample collection but Infection Prevention and Control or other designated staff should be notified, and the Endoscope Reprocessing group and the staff person responsible for reprocessing this duodenoscope should be notified of the reprocessing breach.

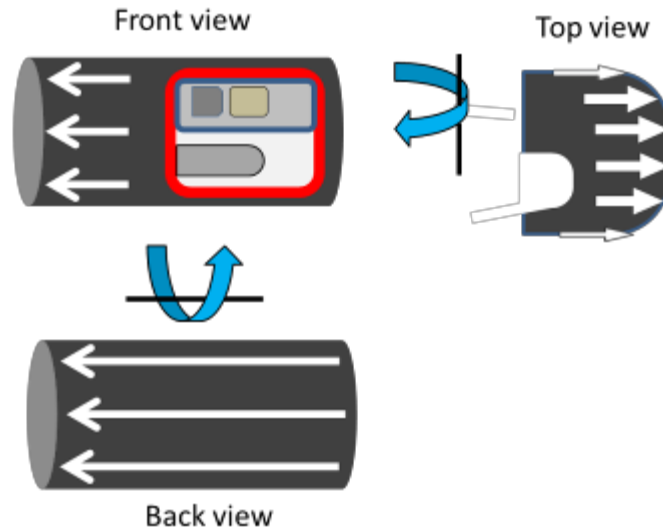
There should also be a review of the reprocessing procedures for the distal end of the duodenoscope, including the elevator lever and elevator recess. The duodenoscope should undergo additional reprocessing (cleaning, inspection, and high level disinfection or sterilization) after sampling. Personnel from responsible departments (e.g., Risk Management and Infection Prevention and Control) should carry out any additional steps necessary after identifying debris on a reprocessed duodenoscope, in accordance with the healthcare facility's written plan.

A5.3.2 Distal cap seams sampling method (note that these steps may be omitted for duodenoscopes with a removable distal cap)

A5.3.2.1 Facilitator: Open a sterile alcohol wipe package.

A5.3.2.2 Sampler: Remove sterile alcohol wipe from package. Wipe exterior of distal end with alcohol wipe. Ensure that the elevator recess and the seams near the elevator recess are not exposed to the alcohol during wiping (refer to Figure 2, below). Wipe away from the elevator recess, taking care to avoid the elevator lever, recess, and the seams between the distal cap and distal end. Allow the alcohol to dry.

Figure 2: Close-up of duodenoscope distal end, and location and direction of alcohol wiping (white arrows) of the exterior of the distal end, and location of seams to swab for sampling (in red). The seams between the distal cap and the distal end may be of variable design with different models of duodenoscopes, and may not extend completely around the elevator recess.



A5.3.2.3 Facilitator: Open the sterile swab package. Open the sterile water container (loosely place the cap back on the sterile water container after the sampler has moistened the swab in sterile water).

A5.3.2.4 Sampler: Remove the sterile swab from the package. Moisten the sterile swab in sterile water. Swab along the seam between the distal cap and the distal end (refer to Figure 2). The distal cap is frequently a plastic material, whereas the distal end is frequently made of metal.

A5.3.2.5 Facilitator: Open the sample collection container and hold the container to allow sampler to break off the tip of the sterile swab into the sample collection container. Alternatively, the facilitator should use sterile scissors to cut off the swab head into the sample collection container. Close the sample collection container.

A5.3.3 Elevator recess sampling method

A5.3.3.1 The design of the elevator recess in current Fujifilm and Olympus duodenoscopes is such that extraction fluid can be applied to the elevator recess and remain within that cavity; in contrast, currently marketed PENTAX duodenoscopes have a hole in the cavity or a removable distal cap. Fluids that are applied to the

elevator recess in PENTAX duodenoscopes will drain (by gravity) out of this cavity. For that reason, the sample container must be held immediately underneath the PENTAX distal end to avoid sample loss. When sampling from the elevator recess of Fujifilm and Olympus duodenoscopes, refer to steps in A5.3.3.2. For PENTAX duodenoscopes with a hole in the elevator recess or a removable distal cap, refer to steps in A5.3.3.3

- A5.3.3.2 Elevator recess sampling method for a duodenoscope with a fixed distal cap with no hole (Fujifilm and Olympus)
- A5.3.3.2.1 Facilitator: Open the package for the sterile pipette or sterile plastic transfer bulb. Open the sterile water container (loosely place the cap back on the sterile water container after the sampler has withdrawn sterile water).
 - A5.3.3.2.2 Sampler: Remove the sterile pipette or sterile plastic transfer bulb from the package and fill with 1 mL of fresh sterile water.
 - A5.3.3.2.3 Facilitator: Lower the elevator lever.
 - A5.3.3.2.4 Sampler: While holding the distal end so that it is parallel to or lying flat on the sterile drape or pad, apply 1 mL of sterile water into the elevator recess with the sterile pipette or plastic transfer bulb.
 - A5.3.3.2.5 Sampler: Use the same pipette or transfer bulb to draw that fluid up and down into pipette or bulb five times.
 - A5.3.3.2.6 The sampler suctions the fluid into the pipette or transfer bulb while facilitator raises the elevator lever. Sampler repeats the previous two steps by applying the fluid into the recess and

drawing the fluid up and down into the pipette or bulb five times.

A5.3.3.2.7 Facilitator: Open the sample collection container (close the container after the sampler has added the sample).

A5.3.3.2.8 Sampler: Use the same pipette or bulb to remove fluid from the elevator recess and transfer the fluid to the sample collection container.

A5.3.3.2.9 Facilitator: Open the package for the sterile elevator cleaning brush. Open the sterile water container (loosely place the cap back on the sterile water container after the sampler has moistened the brush in sterile water).

A5.3.3.2.10 Sampler: Remove the sterile elevator cleaning brush from the packaging and moisten in fresh, sterile water.

A5.3.3.2.11 The sampler brushes the elevator recess while facilitator raises and lowers the elevator.

A5.3.3.2.12 Sampler places brush head over sampling container. Facilitator uses sterile scissors or sterile wire cutters to cut off the entire head of the bristled portion of the brush and allows it to be placed into the sample container.

A5.3.3.2.13 With a new sterile pipette or transfer bulb, repeat steps A5.3.3.2.1 to A5.3.3.2.8.

A5.3.3.3 Elevator recess sampling for a duodenoscope with a hole in the distal cap or with a removable distal cap (PENTAX)

A5.3.3.3.1 Facilitator: Open the package for the sterile pipette or sterile plastic transfer

- bulb. Open the sterile water container (loosely place the cap back on the sterile water container after the sampler has withdrawn sterile water).
- A5.3.3.3.2 Sampler: Remove the sterile pipette or sterile plastic transfer bulb from the package and fill with 1 mL of fresh sterile water
- A5.3.3.3.3 Facilitator: Lower the elevator lever.
- A5.3.3.3.4 While sampler holds the distal end so that it is parallel to the draped counter surface, facilitator places the open sample collection container underneath the distal end of the duodenoscope. Sampler applies 1 mL of sterile water into the elevator recess with the sterile pipette or plastic transfer bulb, and allows that volume to drain into the sample collection container by gravity.
- A5.3.3.3.5 Sampler applies a second 1 mL volume of sterile water to the elevator recess, capturing the volume as it exits the elevator recess or drains through the hole in the back of the cavity and into the sample collection container.
- A5.3.3.3.6 Facilitator raises the elevator lever. Sampler repeats the previous two steps by applying 1 mL of sterile water to the elevator recess twice, allowing the extraction fluid to drain from the cavity by gravity and into the sample collection container.
- A5.3.3.3.7 Facilitator opens the package for the sterile elevator cleaning brush. The sampler removes the sterile elevator cleaning brush from the packaging.

- A5.3.3.3.8 Sampler moistens the sterile elevator sampling brush in fresh, sterile water.
 - A5.3.3.3.9 Sampler brushes the elevator recess while facilitator raises and lowers the elevator.
 - A5.3.3.3.10 Facilitator uses sterile scissors or sterile wire cutters to cut off the entire head of the bristled portion of the brush and place it into the sample container.
 - A5.3.3.3.11 With a new sterile pipette or transfer bulb, repeat steps A5.3.3.3.1 to A5.3.3.3.6.
- A5.3.4 Elevator wire channel sampling method (only for duodenoscopes with an open elevator wire channel)
- A5.3.4.1 Facilitator: Open the packaging for the sterile elevator wire channel washing/flushing/cleaning tube adapter.
 - A5.3.4.2 Sampler removes the sterile elevator wire channel washing/flushing/cleaning tube adapter and attaches it to the elevator wire channel port.
 - A5.3.4.3 Facilitator: Open the packaging for the sterile 5 mL syringe.
 - A5.3.4.4 Sampler: Remove the sterile 5 mL syringe from the packaging and draw up 3 mL of sterile water into the syringe. Attach the syringe to the elevator wire channel washing/flushing/cleaning tube adapter.
 - A5.3.4.5 Facilitator positions the duodenoscope so that it is nearly vertical. Facilitator holds the control handle of the duodenoscope and the syringe (attached to the elevator wire channel adapter) while sampler holds the distal end. Having the facilitator stand on a step stool while holding the control handle may assist in keeping the duodenoscope vertical.
 - A5.3.4.6 Sampler ensures that the sample collection container is at the distal end to allow for collection of the liquid

sample.

A5.3.4.7 Facilitator slowly flushes 3 mL of sterile water into the elevator wire channel. Sampler captures the fluid at the distal end.

A5.3.4.8 While the facilitator continues to hold the duodenoscope, the sampler places the sample collection container on the drape. The sampler then removes the syringe from the elevator wire channel adapter, fills the syringe with air, and re-attaches the syringe to the elevator wire channel adapter. The sampler takes the sample collection container and positions it under the distal end of the duodenoscope.

A5.3.4.9 Facilitator flushes 5 mL of air into the elevator wire channel to evacuate the channel of fluid water. Sampler continues to ensure that the sample collection container is at the distal end to allow collection of the fluid. After the air has been flushed into the channel, the sampler may cap the sample collection container and place it on the sterile drape.

A5.3.4.10 Facilitator removes the elevator wire channel washing/flushing/cleaning tube/adapter from the elevator wire channel port and places the duodenoscope on the sterile drape.

A5.3.5 Instrument channel sampling method

A5.3.5.1 Facilitator opens the packages for two 30 mL syringes. Sampler removes each syringe from the packaging. The facilitator opens the sterile water bottle while sampler fills each syringe with 20 mL of water. The sampler places the syringes on the sterile drape.

A5.3.5.2 Facilitator elevates the control handle of the duodenoscope so that the duodenoscope is nearly vertical. Sampler hands a syringe to the facilitator and holds the distal end over the sample collection container.

A5.3.5.3 Facilitator flushes the instrument channel (via the

biopsy port) with 20 mL of sterile water, which sampler captures in the sample collection container.

A5.3.5.4 Facilitator fills the syringe with air and flushes air into the instrument channel. Any residual fluid is captured in the sample collection container. After the air has been flushed into the channel, the sampler may cap the sample collection container and place it on the sterile drape.

A5.3.5.5 Facilitator places the duodenoscope on the sterile drape. Facilitator opens the sterile instrument channel brush package and sterile scissors package. Sampler removes the sterile instrument channel brush from the packaging.

A5.3.5.6 Facilitator holds the duodenoscope vertically while sampler inserts the sterile instrument channel brush into the biopsy port. Once the brush has been inserted about 3 inches, the sampler transfers the brush handle to the facilitator. The sampler holds the collection container at the distal end to capture any fluid that exits the channel with the brush, making sure not to touch the distal end. The facilitator continues to push the brush through the instrument channel. After the brush head exits the distal tip, the sampler uses sterile scissors or a sterile wire cutter to cut the entire head of the bristled portion of the brush and places it into the sample collection container. The remainder of the brush should be pulled out of the duodenoscope from the biopsy port. Do not attempt to force the brush handle out through the distal end of the duodenoscope. Discard the brush handle appropriately.

A5.3.5.7 Repeat steps A5.3.5.2 to A5.3.5.4.

A5.3.6 Addition of neutralizer solution and transport preparation

A5.3.6.1 Add 45 mL of DE broth or other appropriate neutralizer solution to the sample. Do not allow the duodenoscope to contact the neutralizer solution in the sample container. Accidental immersion of any part of the duodenoscope distal end into the

neutralizer solution necessitates complete reprocessing (cleaning, inspection, and high level disinfection or sterilization) of the duodenoscope after completion of the sampling protocol.

A5.3.6.1.1 Neutralizer solution (e.g. DE broth) is added to facilitate outgrowth of microbes that have been potentially damaged by the reprocessing process.

A5.3.6.2 Tightly close lid of sample container and place sample on ice or ice pack, or in a refrigerator ($2 - 8^{\circ}\text{C} \pm 2^{\circ}\text{C}$) prior to transport to the laboratory for culturing.

A5.3.6.2.1 The volume of fluid in the sample container should be approximately 90 mL (45 mL of DE broth + 40 mL instrument channel sample fluid + 2 mL elevator recess sample + 3 mL elevator wire channel sample where applicable). Some volume loss during sampling (~1 mL) is normal and expected.

A5.3.6.3 Refer to Section A8 for discussion of storage and transport of samples to the microbiology laboratory for culture.

A5.3.7 Sampling of additional channels

A5.3.7.1 Healthcare facilities may also choose to sample additional channels in duodenoscopes, such as the air/water and suction channels by flushing those channels with sampling fluid (sterile water). The volume of flush solution will vary depending on the channel dimensions, and endoscope model-specific connectors may be required for flushing different channels. See Appendix 1 for examples of appropriate flush volumes for various channel dimensions, and contact the device manufacturer to obtain information on connectors.

A6.0 Duodenoscope Handling After Sampling

A6.1 This protocol specifies that sampling should be conducted with sterile water

using sterile implements (e.g., sterile brushes, syringes, etc.) and handled in a manner similar to the rinsing instructions conducted after high level disinfection. The protocol minimizes the risk of contaminating the duodenoscope, and the handling of the duodenoscope during sampling is deemed equivalent to duodenoscope handling during reprocessing. For those reasons, complete reprocessing of the duodenoscope after conducting the sampling described in this protocol is not necessary.

A6.2 Healthcare facilities may choose from any of the three options:

A6.2.1 Option 1: With or without manual cleaning, place the duodenoscope in an automated endoscope reprocessor (AER) and conduct a complete cycle, including any duodenoscope drying steps.

A6.2.2 Option 2: Conduct manual high level disinfection of the device (with or without manual cleaning), including the drying steps after high level disinfection.

A6.2.3 Option 3: Subject the duodenoscope to ethylene oxide sterilization (with or without manual cleaning), if indicated in the duodenoscope labeling.

A6.3 After high level disinfection or sterilization, store the duodenoscope per the policy established in the healthcare facility.

A7.0 Documentation

A7.1 The requisition form for sample submission to the appropriate microbiology staff for culturing should include, but is not limited to, the following information:

A7.1.1 Facility name

A7.1.2 Name and appropriate contact information (e.g., phone number, email address, fax number) of sampling Department/staff requesting the test

A7.1.3 Duodenoscope identifier (model and serial number)

A7.1.4 Sample site (e.g. instrument channel and elevator recess)

A7.1.5 Date and time of sample collection

A7.1.6 Identification of person(s) collecting the sample

A7.1.7 Reason for collecting the sample (e.g., surveillance sampling, re-testing, etc.).

A7.2 The date of the most recent reprocessing cycle and the method employed for reprocessing the duodenoscope should be documented. That information may be included in the requisition form, or may be documented elsewhere.

A8.0 Transport of Endoscope Samples to Microbiology Laboratory

A8.1 Endoscope samples and appropriate documentation should be promptly transferred to the laboratory for culture. Samples should be kept on ice or at 2 - 8°C ($\pm 2^\circ\text{C}$) to avoid overgrowth of low/moderate-concern organisms. When cultured at an on-site laboratory, samples should be cultured within 24 hours of collection. Culture results from samples that have been inappropriately stored (e.g., exceed the maximum storage time or temperature) should be disregarded (except when a high-concern organism is cultured), and additional samples from that duodenoscope should be collected to satisfy the healthcare facility's sampling and culturing policy. Inappropriate storage may result in overgrowth of low/moderate-concern organisms (leading to a false positive result), or result in non-viability of organisms (leading to a false negative result), however, the presence of any number of high-concern organisms would require action.

A8.2 Some healthcare facilities may choose to send the samples out to an Environmental/Reference Laboratory if logistically more feasible. When samples from an endoscope are transported to an off-site laboratory, they must be labeled with biohazard designations and transported within a secondary barrier container with appropriate labeling (e.g., UN3373 [Category B]). Transport according to approved institutional protocols for biohazard transport. When transporting samples to an off-site facility (e.g., Environmental/Reference Laboratory) for culturing, the transport conditions (including time, temperature, and package integrity) must be defined, controlled, monitored, and documented. When the pre-determined transport time and temperature conditions exceed 24 hours at 2 – 8°C, it is recommended to conduct validation testing of transport conditions, where viability/overgrowth of specific organisms under worst-case conditions is assessed. Culture results from samples that have been inappropriately stored (e.g., exceed the maximum pre-determined storage time or temperature) should be disregarded (except when a high-concern organism is cultured), and additional samples from that duodenoscope should be collected to satisfy the healthcare facility's surveillance sampling and culturing policy. Inappropriate storage may result in overgrowth of low/moderate-concern organisms (leading to a false positive result), or result in non-viability of

organisms (leading to a false negative result), however the presence of any number of high-concern organisms would require action.

- A8.3 Do not freeze samples or allow samples to be heated. Extreme temperatures can reduce bacterial viability, resulting in false negatives.

B Culturing Protocol

B1.0 Introduction

- B1.1 Purpose: To provide general recommendations for culturing samples from duodenoscopes.
- B1.2 Scope: The culturing protocol is limited to identifying organisms of concern, some of which have been associated with outbreaks of infections due to contaminated duodenoscopes. Duodenoscope culturing is not intended to identify all microbes that could potentially be found on a duodenoscope, and is not a test for sterility of the device. The culture conditions include an extended incubation period to improve detection of damaged or biofilm-associated microorganisms that may require a longer incubation time to stimulate growth.
- B1.3 The culturing methods described below are intended to detect the most common high-concern organisms on reprocessed duodenoscopes, but these methods will not culture all microbes that could potentially contaminate a duodenoscope and cause infections, e.g., *Pseudomonas fluorescens*. The growth of fastidious organisms such as *Haemophilus* or *Mycobacterium* species will likely not be supported using the conditions described below. The culture conditions may need to be revised if additional types of endoscopes, such as bronchoscopes, are sampled. In the event of an outbreak of infection linked to reprocessed endoscopes, a healthcare facility's culturing protocol may differ significantly from this protocol to maximize the sensitivity of the culturing (i.e., the culturing method may need to be tailored to the suspected causative agent of infection).

B2.0 Safety

- B2.1 Although endoscope samples are not clinical samples and the endoscope has been reprocessed, the endoscope has previously been in contact with a patient and appropriate precautions should be taken. All samples from endoscopes should be considered potentially biohazardous, and therefore the use of standard precautions and appropriate PPE for collecting, handling, and transporting these samples is indicated.

B2.2 Culture of samples may generate aerosols and so must be performed using appropriate containment (e.g. biosafety hood) and PPE.

B2.3 When endoscope samples are transported to an off-site laboratory, they must be labeled with biohazard designations and transported within a secondary barrier container and appropriately labeled (e.g., UN3373; see section A8).

B3.0 Responsibilities

B3.1 Culturing should be conducted by staff who have knowledge and experience with standard microbiological culturing (e.g., clinical diagnostic laboratory staff or commercial laboratory staff with culture expertise).

B3.2 Culturing should be conducted in accordance with the healthcare facility's policies and procedures.

B3.3 Culturing staff are responsible for culturing the endoscope samples in accordance with the facility's surveillance sampling and culturing plan, documenting results, and providing Infection Prevention and Control staff (or other designated Management staff) with interim and final endoscope culturing reports.

B3.4 Infection Prevention and Control staff (or other designated Management staff) are responsible for timely reviewing of endoscope culture reports, alerting responsible department and senior management personnel when culture results exceed acceptable limits, and ensuring remedial action plans are executed.

B4.0 Culturing Proficiency

B4.1 Currently there are no standardized external proficiency testing (EPT) programs for testing laboratories that perform endoscope culturing.

B4.2 Samples from endoscopes are not clinical specimens from patients, they are not used for diagnostic purposes, and they are not used to certify an endoscope as sterile. As such, culturing endoscope samples as a quality indicator of endoscope reprocessing is not subject to CLIA oversight or FDA review.

B4.3 Assessing the adequacy of culture techniques from endoscope samples is entirely voluntary. Facilities that elect to assess the adequacy of culture techniques in their facility may choose to subscribe to an EPT program for quantitative water culturing, or may establish a reciprocal exchange program with another laboratory, in which one laboratory sends a sample with a

known amount of bacteria to a second laboratory for quantitative analysis and interpretation.

B5.0 Culturing Methods

B5.1 Overview: Four options for culturing endoscope samples are discussed below: plating or liquid culture, preceded by filtration or centrifugation. Healthcare facilities should evaluate each option and select the option most appropriate for their facility, based on the needs and resources of the facility, as well as data collected from culturing reports. In general, filtration is preferred to centrifugation because of the potential to lose colony-forming units (CFUs) during centrifugation and supernatant removal.

B5.2 Plating Methods

B5.2.1 Plating with membrane filtration

B5.2.1.1 Materials and equipment

- B5.2.1.1.1 Materials obtained from sampling staff (endoscope sample and sampling documentation)
- B5.2.1.1.2 Sterile membrane filtration apparatus with 0.45 µm filter membrane
- B5.2.1.1.3 Vacuum source
- B5.2.1.1.4 Vortexer
- B5.2.1.1.5 Sterile forceps
- B5.2.1.1.6 Sterile pipette (25 mL or 50 mL)
- B5.2.1.1.7 Blood agar plate
- B5.2.1.1.8 Incubator (optimal temperature for incubation is 35°C to 37°C)

B5.2.1.2 Method for plating with membrane filtration

- B5.2.1.2.1 Document the time and date of filtration.

- B5.2.1.2.2 Aseptically assemble a filtration unit and attach it to a vacuum source. Pre-wet the membrane with an appropriate fluid as needed.
- B5.2.1.2.3 Vortex the endoscope sample for 10 to 20 seconds.
- B5.2.1.2.4 Aseptically remove the swab and brush heads using sterile forceps or aseptically transfer the liquid contents of the container with a sterile pipette to a separate sterile container.
- B5.2.1.2.5 Filter the entire volume of the endoscope sample (~90 mL) through the 0.45 μm filter. Rinse the sides of the filtration unit with an appropriate fluid as needed.
- B5.2.1.2.6 Using sterile forceps remove the filter and place it on the blood agar plate per standard microbiological technique. Take care to gently press the filter down on the plate to ensure contact between the filter and the plate.
- B5.2.1.2.7 Incubate the blood agar plate at 35°C to 37°C (optimally) for a total of 72 hours. Read the plate at 24, 48 and 72 hours. If the plate remains negative at 48 hours of incubation, the duodenoscope may be released from quarantine (if applicable) for further use. Continue to incubate the plate for a full 72 hours. Most cultures will be positive within 24-48 hours. However, some organisms may be less fit due to previous exposure to antibiotics or disinfectants and may require longer growth times. Thus, as a compromise between holding the duodenoscopes longer and recovering the damaged organisms, quarantined

duodenoscopes can be released at 48 hours if the cultures remain negative, but the plates should still be incubated the full 72 hours.

B5.2.1.2.8 Document colony-forming units (CFU) on the blood agar plate at 72 hours, and identify any microbes to the extent necessary to distinguish high-concern organisms from low/moderate-concern organisms. See Section B6.3 for result interpretation.

B5.2.2 Plating with centrifugation

B5.2.2.1 Materials and equipment

B5.2.2.1.1 Materials obtained from sampling staff (endoscope sample and sampling documentation)

B5.2.2.1.2 Vortexer

B5.2.2.1.3 Sterile forceps

B5.2.2.1.4 Sterile 50 mL conical tubes for centrifugation (2 tubes)

B5.2.2.1.5 Centrifuge capable of holding 50 mL conical centrifuge tubes and able to generate 3500-5000 x *g*.

B5.2.2.1.6 Sterile pipettes (25 mL or 50 mL)

B5.2.2.1.7 Sterile Dey-Engley (DE) Broth or other appropriate neutralizer solution (0.1 mL)

B5.2.2.1.8 Blood agar plate

B5.2.2.1.9 Sterile spreader (e.g., "hockey stick")

B5.2.2.1.10 Incubator (optimal temperature for incubation is 35°C to 37°C)

- B5.2.2.2 Method for plating with centrifugation
- B5.2.2.2.1 Document the time and date of sample processing.
 - B5.2.2.2.2 Vortex the endoscope sample for 10 to 20 seconds.
 - B5.2.2.2.3 Aseptically remove the swab and brush heads using sterile forceps or aseptically transfer the full liquid contents of the container with a sterile pipette to a separate sterile container.
 - B5.2.2.2.4 Equally distribute the sample to two sterile 50 mL conical tubes for centrifugation.
 - B5.2.2.2.5 Centrifuge the samples at 3500-5000 *x g* for 10-15 minutes.
 - B5.2.2.2.6 Carefully remove and discard the supernatant from each tube. To one of the tubes, resuspend the cells in 0.1 mL of DE broth or other appropriate neutralizer solution. Transfer the resuspension to the second tube and resuspend the cells in the same solution.
 - B5.2.2.2.7 Spread plate the entire volume (0.1 mL) on a blood agar plate.
 - B5.2.2.2.8 Incubate the plate at 35°C to 37°C (optimally) for a total of 72 hours. Read the plate at 24, 48 and 72 hours. If the plate remains negative for growth at 48 hours of incubation, the duodenoscopes may be released from quarantine (if applicable) for further use. Continue to incubate the plate for a full 72 hours. Most plates will be positive within 24-48 hours. However,

some organisms may be less fit due to previous exposure to antibiotics or disinfectants and may require longer growth times. Thus, as a compromise between holding the duodenoscopes longer and recovering the damaged organisms, quarantined duodenoscopes can be released at 48 hours if the cultures remain negative, but the plates should still be incubated the full 72 hours.

- B5.2.2.2.9 Document colony-forming units (CFU) on the blood agar plate at 72 hours, and identify any microbes to the extent necessary to distinguish high-concern organisms from low/moderate-concern organisms. See Section B6.3 for result interpretation.

B5.3 Liquid Culture Methods

B5.3.1 Liquid culture with membrane filtration

B5.3.1.1 Materials

- B5.3.1.1.1 Materials obtained from sampling staff (endoscope sample and sampling documentation)
- B5.3.1.1.2 Sterile membrane filtration apparatus with 0.45 µm filter membrane
- B5.3.1.1.3 Vacuum source
- B5.3.1.1.4 Vortexer
- B5.3.1.1.5 Sterile forceps
- B5.3.1.1.6 Sterile pipette (25 mL or 50 mL)
- B5.3.1.1.7 100 mL sterile Dey-Engley (DE) Broth or other appropriate neutralizing media in sterile culture flask. Note: this volume

may be decreased if the entirety of the filter can be immersed in broth.

B5.3.1.1.8 Incubator (optimal temperature for incubation is 35°C to 37°C)

B5.3.1.2 Method

B5.3.1.2.1 Document the time and date of filtration.

B5.3.1.2.2 Aseptically assemble a filtration unit and attach it to a vacuum source. Pre-wet the membrane with an appropriate fluid as needed.

B5.3.1.2.3 Vortex the endoscope sample for 10 to 20 seconds.

B5.3.1.2.4 Aseptically remove the swab and brush heads using sterile forceps, or aseptically transfer the liquid contents of the container with a sterile pipette to a separate sterile container.

B5.3.1.2.5 Filter the entire sample volume through the 0.45 µm membrane filter. Rinse the sides of the filtration unit with an appropriate fluid as needed.

B5.3.1.2.6 Using sterile forceps remove the filter and place it in the culture flask containing 100 mL fresh DE broth or other appropriate neutralizing media. The volume of broth may be decreased to a level that would still allow complete immersion of the filter.

B5.3.1.2.7 Incubate the broth culture at 35°C to 37°C (optimally) for a total of 72 hours. Monitor for growth at 24, 48 and 72 hours.

B5.3.1.2.8 Identify and document no growth /

positive growth using standard methods (e.g., turbidity, spectrophotometric readings, etc.).

B5.3.1.2.9 If growth is detected in the broth, perform a Gram stain of the broth and subculture the broth (e.g., to blood agar and MacConkey plates), streaking for isolation. Incubate the plates at 35°C to 37°C (optimally) approximately 16 hours (overnight) and up to 72 hours. Report the Gram stain as an interim report with further results to follow and indicate that the final report will be generated after incubating plates for 72 hours.

B5.3.1.2.10 Any microbial growth should be identified (at a minimum) to the extent necessary to differentiate high-concern organisms from low/moderate-concern organisms. See Section B6.4 for result interpretation.

B5.3.2 Liquid culture with centrifugation

B5.3.2.1 Materials

B5.3.2.1.1 Materials obtained from sampling staff (endoscope sample and sampling documentation)

B5.3.2.1.2 Vortexer

B5.3.2.1.3 Sterile forceps

B5.3.2.1.4 Sterile 50 mL conical tubes for centrifugation (2 tubes)

B5.3.2.1.5 Centrifuge capable of holding 50 mL conical centrifuge tubes and able to generate 3500-5000 x *g*.

B5.3.2.1.6 Sterile pipettes (25 mL or 50 mL, and a

- small volume pipette (1 mL to 5 mL)
- B5.3.2.1.7 4 to 10 mL sterile Dey-Engley (DE) Broth or other appropriate neutralizing media
- B5.3.2.1.8 Sterile culture flask for 4 to 10 mL volume culture
- B5.3.2.1.9 Incubator (optimal temperature for incubation is 35°C to 37°C)
- B5.3.2.2 Method
 - B5.3.2.2.1 Document the time and date of sample processing.
 - B5.3.2.2.2 Vortex the sample for 10 to 20 seconds.
 - B5.3.2.2.3 Aseptically remove the swab and brush heads using sterile forceps, or aseptically remove the liquid contents of the container with a sterile pipette.
 - B5.3.2.2.4 Equally distribute the sample to two sterile 50 mL conical tubes for centrifugation.
 - B5.3.2.2.5 Centrifuge the samples at 3500-5000 x *g* for 10-15 mins.
 - B5.3.2.2.6 Carefully remove and discard the supernatant from each tube. To one of the tubes, resuspend the cells in 1 mL of DE broth or other appropriate neutralizing media. Transfer the resuspension to the second tube and resuspend the cells in the same solution.
 - B5.3.2.2.7 Transfer the entire sample to the sterile culture flask and add remaining DE broth or other appropriate neutralizing media, for a total volume

of between 4 to 10 mL.

- B5.3.2.2.8 Incubate the broth culture at 35°C to 37°C (optimally) for a total of 72 hours. Monitor for growth at 24, 48 and 72 hours.
- B5.3.2.2.9 Identify and document no growth / positive growth using standard methods (e.g., turbidity, spectrophotometric readings, etc.).
- B5.3.2.2.10 If growth is detected in the broth, perform a Gram stain of the broth and subculture the broth (e.g., to blood agar and MacConkey plates), streaking for isolation. Incubate the plates at 35°C to 37°C (optimally) overnight and up to 72 hours. Report the Gram stain as an interim report with further results to follow and indicate that the final report will be generated after incubating plates for 72 hours.
- B5.3.2.2.11 Any microbial growth should be identified (at a minimum) to the extent necessary to differentiate high-concern organisms from low/moderate-concern organisms. See Section B6.4 for result interpretation.

B5.4 Controls

- B5.4.1 Culture media should either be appropriately tested or should be supplied with documentation from the media manufacturer that appropriate control testing and/or analysis was performed. Examples of control testing include testing to demonstrate sterility of the media (negative control) and testing to demonstrate indicator organisms will grow (positive control). Clinical laboratories should follow standard regulatory requirements for media controls.
- B5.4.2 All quantitative pipettes should be calibrated yearly and all incubators should have daily temperature monitoring. Clinical laboratories should follow standard regulatory requirements for

pipette calibrations.

B6.0 Examples of Microbial Limits

B6.1 Results Overview: Table 1 below provides an overview of examples of microbial limits for endoscope culturing. As discussed in more detail below, limits for low/moderate-concern organisms should be established by healthcare facilities based on data from culturing reports, but the examples below can be used when first initiating endoscope sampling and culturing in a healthcare facility.

Table 1: Interpretation of culture results on blood agar plate at 72 hours.

Blood Agar Plate Results				
	>100 CFU	11 - 100 CFU	1 - 10 CFU	0 CFU
High-concern organisms	Action	Action	Action	No action
Low/moderate-concern organisms	Action	Alert	No action	No action
Liquid Culture Results				
	Growth detected		No growth detected	
High-concern organisms	Action		No action	
Low/moderate-concern organisms	Modified Action		No action	

B6.2 Potential Response Overview: Table 2 provides an overview of potential responses following culture results. See Section B6.3 for additional details following plating, and Section B6.4 for additional details following liquid culture.

Table 2: Potential Responses following culture results

Potential Responses	Culture Results			
	Action	Alert	Modified Action	No Action
Remove duodenoscope from use.	X		X	
Conduct a risk/safety management response including potential patient notification and follow-up.	X			
Review reprocessing practices and re-train staff as needed.	X	X		
Review sampling and culturing procedures and	X	X		

re-train appropriate staff, as needed.				
Repeat reprocessing, as needed.	X		X	
Repeat sampling, and apply samples to blood agar plates. Determine next response based on blood agar plate results.			X	
Repeat sampling, and only return the duodenoscope to use if culture results are acceptable and if reprocessing errors have been corrected, if needed.	X			
Duodenoscope return and other investigative activity (e.g., patient follow-up) by the appropriate staff within the healthcare facility, as needed.	Repeat positive cultures that are not alleviated after review and correction (as necessary) of reprocessing and sampling/culturing protocols			

B6.3 Results Interpretation – Plating Results

B6.3.1 Undefined organisms

B6.3.1.1 The protocol is not intended to culture all organisms as it is focused on the recovery of bacteria associated with infectious outbreaks. The finding of any other organism not specifically listed in this document as low/moderate or high-concern organisms should be classified into the appropriate category by appropriate staff within the healthcare facility. It may not always be possible for the laboratory to enumerate growth on a plate (i.e. overgrowth of a fungal microorganism) or to identify a microorganism to a genus or species level (i.e. fungi). In these cases the presence of the microorganism should be reported with minimal identification required (i.e. yeast present, filamentous fungi present). Appropriate staff within the healthcare facility should determine the significance of these findings.

B6.3.2 High-concern organisms

B6.3.2.1 One or more colonies of a high-concern organism exceeds the microbial limit for endoscope samples and requires action.

B6.3.2.2 Isolation of a high-concern organism from a reprocessed endoscope warrants removal of the endoscope from use. Reprocessing practices should

be reviewed to identify potential improvements in the process and to verify that the endoscope is being processed in accordance with professional guidelines and the manufacturer's instructions for use. The endoscope should be reprocessed (incorporating reprocessing improvements and corrections, if applicable), and the endoscope should be sampled and cultured before the next patient use. The endoscope may be returned to use only if repeat culture is negative (or ≤ 10 CFU of low/moderate-concern organisms) and no protocol breaches in reprocessing were identified. If cultures are repeatedly positive for high-concern organisms, the healthcare facility should consider returning the duodenoscope to the manufacturer.

B6.3.2.3 The healthcare facility's written plan should include a risk/safety management response, including potential patient notification and follow-up when high-concern organisms are cultured from reprocessed endoscopes. Refer to CDC's Patient Notification Toolkit (<https://www.cdc.gov/injectionsafety/pntoolkit/index.html>) for additional information.

B6.3.3 Low/moderate-concern organisms

B6.3.3.1 The clinical relevance of the growth of low/moderate-concern organisms from duodenoscopes is not clear. There are no standard limits for low/moderate-concern organisms. The example limits for these organisms (Table 1, below) represent expert opinion. A healthcare facility's own experience with surveillance sampling and culturing should further inform their limits for low/moderate-concern organisms.

B6.3.3.2 Limits for low/moderate-concern organisms should be established by responsible personnel within the healthcare facility based on the healthcare facility's own culturing data. Facilities can monitor the levels of these bacteria within the first several months of surveillance testing until consistent results are observed to develop an expected baseline for those organisms. Examples of limits for

low/moderate-concern organisms are provided in Table 1 (below) and in the following sections, but should be considered general guidelines. Thresholds for action, alert, or no action can be adjusted from the examples provided in Table 1 based on the healthcare facility's data.

B6.3.3.3 The presence of low/moderate-concern organisms can be a marker for problems with cleaning, storage and handling of these devices, contamination during sampling or processing of specimens, or defects in the device. Low/moderate-concern organisms may be present on a reprocessed endoscope due to contamination during sampling and culturing (from coughing or talking), or they may have been introduced to the device during the clinical procedure and survived the reprocessing process. Facilities with duodenoscopes that consistently grow low/moderate-concern organisms should consider reviewing their sampling and culturing practices for potential sources of contamination and reviewing their protocols for reprocessing and storage of these devices. If individual duodenoscopes consistently grow low/moderate-concern organisms, consideration should be given to returning the device to the manufacturer for evaluation and repair, if necessary. Examples of limits for low/moderate-concern organisms on a blood agar plate are provided below:

B6.3.3.4 ≤ 10 CFU low/moderate-concern organisms: No action

B6.3.3.5 11 to 100 CFU low/moderate-concern organisms:
Alert

B6.3.3.5.1 The clinical relevance of this finding is not clear and reprocessing the device prior to use might not be required. When moderate-concern oral microorganisms are detected, it is recommended that endoscope reprocessing staff review reprocessing methods to verify that the endoscope is being processed in accordance with professional guidelines and the

manufacturer's instructions for use, and review the sampling method to minimize the potential for contaminating endoscope samples. When low-concern skin microorganisms are detected, it is recommended that sampling staff review sample collection procedures, and culturing staff review the culturing protocol to identify the potential for contamination during the process.

B6.3.3.6 >100 CFU low/moderate-concern organisms: Action

B6.3.3.6.1 High levels of low-concern organisms may be indicative of inadequate reprocessing and/or damage to the endoscope. In addition to reviewing endoscope reprocessing and sampling/culturing protocols, healthcare facilities may also choose to remove the endoscope from use, reprocess the endoscope, and conduct additional sampling and culturing of the device before the next patient use. The endoscope may be returned to use if repeat culture is negative (or ≤ 10 CFU of low/moderate-concern organisms) and no reprocessing protocol breaches were identified.

B6.4 Results Interpretation - Liquid culture

B6.4.1 Negative growth: No action

B6.4.2 Positive growth – high-concern organism:

B6.4.2.1 Isolation of a high-concern organism from a reprocessed endoscope warrants removal of the endoscope from use. Reprocessing practices should be verified to confirm that the endoscope is being processed in accordance with professional guidelines and the manufacturer's instructions for use. The endoscope should be reprocessed (incorporating

reprocessing improvements and corrections, if applicable), and the endoscope should be sampled and cultured before the next patient use. The endoscope may be returned to use only if repeat culture is negative (or ≤ 10 CFU of low/moderate-concern organisms) and no protocol breaches in reprocessing were identified. If cultures are repeatedly positive for high-concern organisms, the healthcare facility should consider returning the duodenoscope to the manufacturer.

B6.4.2.2 The healthcare facility's written plan should include a risk/safety management response, including potential patient notification and follow-up when high-concern organisms are cultured from reprocessed endoscopes. Refer to CDC's Patient Notification Toolkit (<https://www.cdc.gov/injectionsafety/pntoolkit/index.html>) for additional information.

B6.4.3 Positive growth – low/moderate-concern organism:

B6.4.3.1 Liquid cultures positive for low/moderate-concern organisms warrant modified action. The clinical relevance of the growth of low/moderate-concern organisms from duodenoscope cultures is not clear; however, the presence of these organisms can be a marker for problems with cleaning, storage and handling of these devices, contamination during sampling or processing of specimens, or defects in the device. Low/moderate-concern organisms may be present on a reprocessed endoscope due to contamination during sampling and culturing (from coughing or talking), or they may have been introduced to the device during the clinical procedure and survived the reprocessing process. Facilities with duodenoscopes that consistently grow low/moderate-concern organisms should consider reviewing their sampling and culturing practices for potential sources of contamination and reviewing their protocols for reprocessing, handling and storage of these devices. If individual duodenoscopes consistently grow low/moderate-concern organisms, consideration should be given to returning the device to the manufacturer for evaluation.

- B6.4.3.2 Distinguishing whether a duodenoscope had <10 CFU or > 100 CFU of a low/moderate-concern organism is not possible when utilizing liquid cultures. In an abundance of caution, the endoscope should be reprocessed and undergo additional sampling and culturing on plates (as described in one of the two methods in section B5.2). Any additional action should be determined from the culture results of the second sample.

B7.0 Result Reporting

- B7.1 If any growth is observed (on plates or in broth), culturing staff should immediately conduct Gram staining and other identification methods to identify potential high-concern organisms. The results of the culture and Gram stain should be provided as an interim report to the Infection Prevention and Control staff or other designated staff within the healthcare facility, as determined by the facility's reporting plan.
- B7.2 Final results at 72 hours should be documented and provided to appropriate staff within the healthcare facility.
- B7.3 Documentation of relevant culturing information should conform with local regulations, quality management policies, and be traceable within the culturing facility's documents. Examples of relevant information include the following:
 - B7.3.1 All of the information in the requisition form for sample submission (see Section A7)
 - B7.3.2 Sample acquisition date and time (date and time when sample was received in laboratory)
 - B7.3.3 Identification of laboratory staff member receiving the sample, or equivalent information to document receipt of sample
 - B7.3.4 Leaking sample containers or improper sample transport conditions
 - B7.3.5 Date and time of sample culturing
 - B7.3.6 Identifier of culturing staff (e.g., initials)
 - B7.3.7 Culturing method

- B7.3.8 Growth results at 24, 48, and 72 hours
- B7.3.9 Gram stain results at 24, 48, and 72 hours, if applicable
- B7.3.10 Organism identification and identification method, if applicable
- B7.3.11 Date results were sent to Infection Prevention and Control staff or other designated Management staff
- B7.3.12 Identification of Infection Prevention and Control staff member (or other designated Management staff) receiving results, or equivalent to track receipt of culture results

Appendix 1 Suggested Sample Collection Volumes for Flexible Endoscopes

Channel dimensions*		Channel volume (mL): ¹	Sample flush volume (mL) ²	Brush ³
Inner diameter (mm)	Length (mm)			
Colonoscopy example: Entire suction channel 3.7 mm	3800 mm	40.6 mL	~ 120 mL	Yes
Colonoscopy example: Auxiliary water channel 1.5 mm	1600 mm	2.9 mL	~ 10 mL	No
Colonoscopy example: Air/water channel (joined) 1 mm	3800 mm	11.9 mL each 23.8 total	~ 40 mL for each channel	No
Gastroscope example: Entire suction channel 2.8 mm	2500 mm	15.4 mL	~ 60 mL	Yes
Duodenoscopy example: Entire suction channel 3.7 mm	3000 mm	32.26 mL	~ 100 mL	Yes
Bronchoscope example: Entire suction channel 2 mm	870 mm	2.73 mL	~ 8 mL	Yes

* These are approximate dimensions only and are used for example calculation purposes only.

¹ Calculate channel volume: $\pi \times r^2 \times h$; where r= radius (diameter in cm/2), h = channel length (cm) (Note: the channel dimensions in mm need to be converted to cm i.e. mm/10).

An alternative way to determine the channel volume is to completely fill the channel with water and then measure the volume of the fluid when it is flushed out into a measuring cylinder.

² Ideally, the sample collection volume used to flush the endoscope channel should be approximately three times the channel volume to ensure adequate sample collection.

³ If a brush is available for the channel, then the sample should be collected by the “flush-brush-flush” method. As such, the total sample flush volume is divided between the two flush steps (e.g. if the sample flush volume is 40 mL then the first flush would be 20 mL followed by the brush step and the second flush would be 20 mL where all the fluid flushed as well as the brush head are collected in the same sterile container).

Appendix 2 – Duodenoscope Photographs

The photographs included below are meant to illustrate duodenoscope design features and accessories used in duodenoscope sampling, and are not intended to be used as a training aid for aseptic handling of duodenoscopes.

Elevator lowered



Elevator half-way up



Elevator raised



A.5.3.2.2 – Wiping the distal end with alcohol.



A.5.3.2.4 – Swabbing along the seam between the distal cap and the distal end



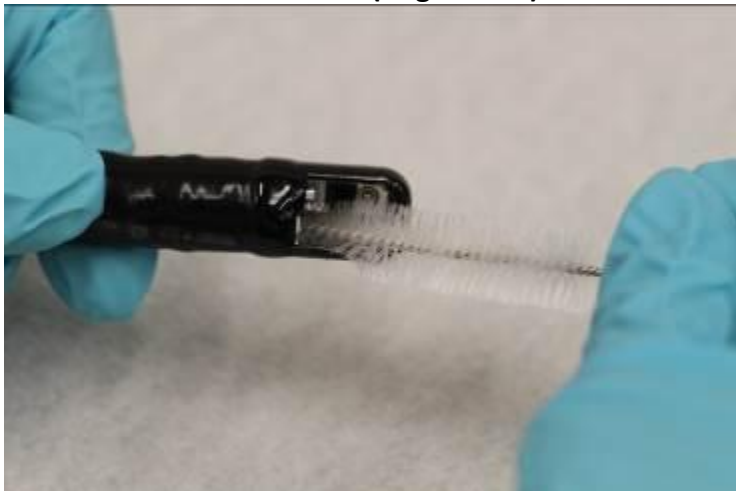
A.5.3.3.2.4 Elevator recess flush – elevator down



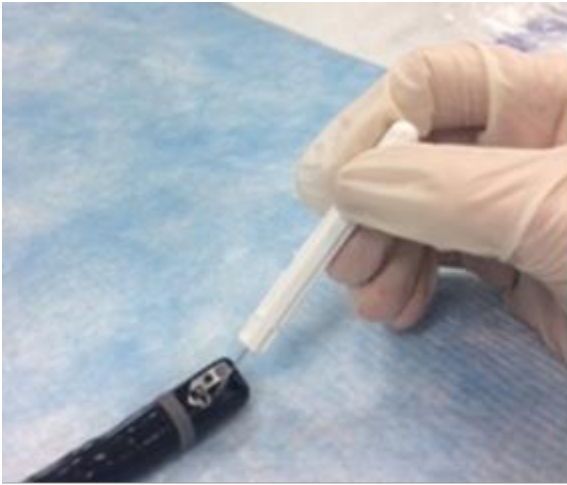
A.5.3.3.2.6 Elevator Recess flush – elevator up



A.5.3.3.2.11 Elevator brush (large brush)



A.5.3.3.2.11 Elevator brush (small brush)



Flushing fluid into the instrument channel (A5.3.5.3)



Flushing fluids into the elevator wire channel (A5.3.4.7)



Keeping duodenoscope vertical when flushing fluids through channels to distal end (A5.3.5.6)



A5.3.5.6 Brush Instrument Channel



A5.3.5.6 Instrument channel brush exiting distal end



Sample container with liquid extract and swab and brush heads (prior to A5.3.6)



Sample container after addition of Dey-Engley broth (after A5.3.6.1)

