Comparison of Two Culture Methods for Use in Assessing Microbial Contamination of Duodenoscopes

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Recent outbreaks of carbapenem-resistant *Enterobacteriaceae* infections associated with duodenoscopes used for endoscopic retrograde cholangiopancreatography have highlighted the challenge of cleaning and high-level disinfection of these instruments. The Food and Drug Administration has suggested that duodenoscope surveillance by microbiological culturing, along with strict adherence to reprocessing protocols, may help reduce the risk of duodenoscope-associated infection transmission. We developed and validated an effective, user-friendly duodenoscope sampling and culture protocol and compared its performance to the interim Centers for Disease Control and Prevention–recommended guidelines. Our protocol resulted in a 65% recovery rate for Gram-negative organisms, demonstrating a 2-fold increased recovery rate compared to the CDC method. The implementation of this protocol may increase the feasibility of duodenoscope surveillance for microbiology laboratories and endoscopy departments.

Endoscopic retrograde cholangiopancreatography (ERCP) is used as a diagnostic and therapeutic tool for many pancreatic and biliary diseases, providing a less invasive treatment option than surgery. There are currently more than 500,000 ERCP procedures performed in the United States annually (1). Duodenoscopes used for ERCP have been linked to serious postprocedure infections, including at least 11 clusters of antibiotic-resistant *Enterobacteriaceae* infections over the past 7 years (2–11). The Food and Drug Administration (FDA) has received notification of 142 cases of patient infection or exposure from reprocessed duodenoscopes since 2010 (12).

Epidemiological investigation of recent ERCP-associated antibiotic-resistant *Enterobacteriaceae* outbreaks revealed that many were not caused by reprocessing deficiencies or detectable device defects (8, 11, 13). Instead, these clusters have been attributed to the complex design of the duodenoscope, which includes an instrument/suction channel with a cantilevered elevator mechanism that allows for the manipulation of equipment during procedures (12, 13). This elevator mechanism contains microscopic crevices that can retain organic debris and fluid despite brushing and cleaning prior to high-level disinfection. As a result, the FDA has recommended supplemental measures to the manufacturers’ recommendations as a means to help reduce the risk of infection transmission. These include microbial culturing, ethylene oxide sterilization, use of liquid chemical sterilant processing, and repeat high-level disinfection (14).

Performing surveillance cultures of endoscopes is a controversial topic in endoscopy infection control but is promoted by some gastroenterological societies as a quality-control marker of the adequacy of cleaning and disinfection in an endoscopy unit and to monitor the integrity of an endoscopy (15–21). The Gastroenterological Society of Australia and the European Society of Gastroenterology (ESGE)–European Society of Gastroenterology and Endoscopy Nurses and Associates (ESGENA) have published formal guidelines for endoscope surveillance (18, 21). Additionally, the Emergency Care Research Institute recently published a high-priority hazard report recommending culturing of duodenoscopes as a key step in reducing carbapenem-resistant *Enterobacteriaceae* infections (22), and the FDA has recommended supplemental measures to enhance reprocessing with microbiological culture as one of the suggested measures (14). While the Centers for Disease Control and Prevention (CDC) published interim guidelines for duodenoscope culturing in 2015 (23), these have not been rigorously investigated, and the performance characteristics are unknown.

We developed and validated an alternate duodenoscope sampling and culture protocol and compared its performance to the CDC-recommended method. Our primary objectives were to develop an effective, user-friendly method and to understand limitations in sample collection and culture.

**MATERIALS AND METHODS**

Soiling of the ERCP duodenoscope. Two Olympus TJF duodenoscopes (model 160VF and model Q180V) were used for culture validation testing in the laboratory. Duodenoscopes were reprocessed between tests by the endoscopy department at LDS Hospital in Salt Lake City, Utah, following standard operating procedures. Artificial test soil plus mucin (ATS-M) (Healthmark) was rehydrated with sterile water following manufacturer specifications. A mixed culture ATS-M inoculum was prepared with approximately 5 × 10^8 CFU/ml of each of the following clinical isolates: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecium*. These organisms were selected because they are considered high-concern or indicator organisms. Serial dilutions of 10^-2, 10^-4, 10^-5, and 10^-6 of ATS-M inoculum were generated and, along with the original 5 × 10^8 concentration, used to artificially contaminate the duodenoscopes. The elevator mechanism at the distal end of the endo-
scope was partially raised to a 90-degree angle. Then, 100 μl of ATS-M inoculum was spotted above and below the elevator mechanism and allowed to dry for 30 min, based on manufacture specifications (24). Two methods of endoscope sampling were evaluated for percent recovery and ease of use. Duodenoscopes were also cultured before inoculation and after reprocessing as controls.

**Modified ESGE protocol for duodenoscope sampling.** We used a modified version of the ESGE guidelines (18) but sampled only the instrument channel (Fig. 1). Briefly, 5 ml of sterile saline (0.9%) was flushed through the instrument channel via the instrument port and collected in a sterile 15-ml conical tube. The elevator mechanism and lens face were then vigorously swabbed with a Floqswab (COPAN), being sure to swab both over and under the elevator mechanism as it was set in its open and closed positions. The swab was broken off below the handling point into the 5 ml of sterile saline previously flushed through the instrument channel and vortexed for 2 min in 10-s bursts. Samples were diluted and plated in triplicate onto tryptic soy agar (TSA) with 5% sheep blood and incubated at 35°C for 24 h. Five experiments (three with model 160VF and two with model Q180V) were performed for each dilution, for a total of 25 samplings using this collection method.

**Interim CDC protocol for duodenoscope sampling.** The CDC interim sampling and quantitative culturing guidelines were followed, and three experiments (two with model 160VF and one with model Q180V) were performed for each dilution, for a total of 15 samplings. Briefly, the elevator mechanism and lens face at the distal end of the duodenoscope was brushed with a duodenoscope channel-opening brush (Olympus MH-507) with the mechanism in both the lowered and raised positions. The brush was placed into sterile phosphate-buffered saline with 0.02% Tween 80 (PBST; Teknova P3875) and vortexed for 2 min in 10-s bursts. Then, 50 ml of sterile water was flushed through the instrument channel via the instrument port and collected in a sterile 50-ml conical tube. The brush and channel fluids were processed separately by centrifuging at 4,200 × g for 15 min and resuspended in 1 ml of either PBST or sterile water, respectively. Samples were diluted and plated in triplicate onto TSA with 5% sheep blood and incubated at 35°C for 24 h. CFU counts from the brush and fluid were combined to calculate the CFU per scope.

**Determining sampling efficiency.** The four bacterial species in the ATS-M inoculum are phenotypically distinct, enabling all four species to be identified morphologically on TSA with 5% sheep blood and counted individually. The CFU/ml count from all duodenoscope-collected fluid was determined for each of the four bacterial species and used to calculate the CFU per scope. Additionally the ATS-M inoculum was diluted, plated onto TSA with 5% sheep blood, and incubated at 35°C for 24 h to obtain the accurate CFU/ml. This value was used to calculate the CFU spotted on the duodenoscope. The CFU per scope was compared to CFU spotted to determine sampling efficiency. ATS-M without bacteria was cultured as a negative control. Student’s t test was used to compare sampling efficiency between sampling methods.
RESULTS
To compare the sampling efficiency of the modified ESGE protocol (MEP) and CDC protocol, we calculated that the distal end of duodenoscopes had been spotted with an average of 10, 100, 10³, and 10⁷ CFU of mixed culture. Sampling duodenoscopes with either the MEP or CDC sampling method indicated a similar percent recovery when comparing the 160VF and Q180V duodenoscope models (data not shown). The similarity in recovery is unsurprising, as both sampling methods focus on the instrument channel and distal end elevator, which are structurally similar between models. Sampling was not performed on the open guide wire channel which is found in the 160VF model but not Q180V model. Data from the 160VF and Q180V were combined for comparison of the MEP and CDC sampling methods.

Ten CFU/scope was found to be below the level of accurate detection for both protocols, as the agar plates frequently showed no growth after sampling (CDC, 4 of 9; MEP 5 of 15). At 100 CFU/scope, the percent recovery of Gram-negative organisms was significantly enhanced (P = 0.0009) using the MEP (64.1%) compared to the CDC protocol (32.9%) but was equivalent for Gram-positive organisms, with approximately 60% recovery for both methods. At 10⁷ CFU/scope, the recovery of Gram-negative organism increased for both protocols; however, a statistical difference (P = 0.03) between percent recovery with MEP (80.6%) and CDC (47.4%) remained. Again, recovery of Gram-positive organisms remained consistent for both protocols at approximately 60% (Fig. 2).

Focusing on endoscopes soiled with 100 CFU, we examined the percent recovery of individual bacterial species. Of the four bacterial species, recovery of P. aeruginosa was greatest using MEP, followed by similar rates of both K. pneumoniae and E. faecium. E. coli had the lowest recovery rate of the four organisms tested (Table 1). Using the CDC protocol, the percentage of P. aeruginosa recovered was highest of the spotted Gram-negative organisms but was 34% less than the MEP protocol. Recovery of K. pneumoniae and E. coli was lowest using the CDC protocol. The percent recovery of E. faecium was similar by both protocols (Table 1).

We observed that the brush recommended by the interim CDC protocol did not completely reach the entire surface of the elevator in either the raised or the lowered position. To visually compare the efficacy of the brush versus the Floqsawab (Copan) at sampling the elevator mechanism, we coated the distal end of an ERCP duodenoscope with GlitterBug potion (Brevis) before sampling and after application of the swab or brush. The residual GlitterBug coating the elevator mechanism was qualitatively lower by visual inspection after using the flocked swab compared to the brush (Fig. 3). The flocked swab also proved to be simple to snap off into the collection vial, minimizing risk of contaminating the culture.

DISCUSSION
The American Society of Microbiology Public and Scientific Affairs Board Committee on Laboratory Practices has recommended that clinical microbiology labs do not perform routine surveillance of duodenoscopes due to a lack of validation and inappropriateness of techniques, equipment, and expertise for a clinical microbiology lab (25). Due to uncertainties surrounding the optimal culturing methods, this study compared the performance of a modified ESGE sampling and culturing method to the CDC interim protocol. While both methods demonstrated qualitative recovery of spiked organisms, the MEP method demonstrated several advantages over the interim CDC sampling protocol and may increase the feasibility of obtaining qualitative duodenoscope surveillance cultures at endoscopy departments.

Sampling using the MEP led to a 2-fold increase in recovery of Gram-negative organisms compared to the CDC protocol. This higher recovery rate is likely due to the use of the flocked swab for collecting samples from under and around the elevator mechanism as opposed to use of the Olympus cleaning brush. Flocked swabs are designed to capture and release organisms, and they are able to fit under the raised elevator mechanism more efficiently than the recommended cleaning brush. The bristles do not extend around the head of the brush, limiting complete sample collection in tight spaces. The brush is able to fit more effectively into the instrument channel for which it was designed. However, the elevator mechanism has been identified as the likely structure for reprocessing failures, making it more likely than the channel to harbor organisms after an unsuccessful cleaning and reprocessing. Olympus has recently designed a smaller brush for improved elevator cleaning to address this problem.

<table>
<thead>
<tr>
<th>Organism</th>
<th>% recovery ± SD by protocol</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>MEP</td>
<td>CDC</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>80.3 ± 23.5</td>
<td>46.2 ± 12.6</td>
</tr>
<tr>
<td>E. coli</td>
<td>46.0 ± 13.0</td>
<td>25.6 ± 7.8</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>66.0 ± 9.7</td>
<td>32.1 ± 3.2</td>
</tr>
<tr>
<td>E. faecium</td>
<td>67.2 ± 15.6</td>
<td>60.2 ± 4.2</td>
</tr>
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During the evaluation, we also observed instances in the CDC protocol where contamination could be introduced into the sampling fluid. The use of a 50-ml flush for the instrument channel requires two people to complete the protocol, as a 60-ml syringe is too cumbersome to use while also holding the scope and collecting the fluid. The large volume combined with the need to coordinate collection between two individuals may increase the possibility of spillage or overflow as the fluid is being passed into the channel. An advantage of small flush volume of the MEP is that a 5-ml syringe allows for single-person collection when necessary, with minimal contamination risk.

Another potential limitation to the CDC protocol that we observed was handling of the Olympus brush following use, as the entire brush is dropped into the collection fluid, including the handle that had been held during collection. The protocol requires that sterile gloves must be worn; however, the collector has likely been handling the collection cup, endoscope, and other items and may introduce contaminants into the sample fluid from the brush itself. The flocked swab prevents this possibility, as it is broken off into the sample fluid below the handling point. This also allows for the use of nonsterile gloves, which is more realistic for completing sampling in an endoscopy department instead of in a dedicated microbiology laboratory.

There are also limitations to the MEP and this study. This investigation did not include soiling of the instrument channel, which could also contain microbial contamination postprocedure. The larger flush volume used in the CDC protocol may be more efficient at removing contamination from the channel. Therefore, the CDC protocol may yield a higher percent recovery under actual in-use conditions than we report here. The ESGE-ESGENA guidelines suggest neutralizer as an additive to the sterile saline to neutralize any chemicals remaining from reprocessing that may inhibit microbial growth. Further testing should be done to determine if the addition of neutralizer increases percent recovery, which would be necessary for scopes contaminated with very low levels of organisms. Additionally, this study was completed by trained microbiologists in a laboratory environment using loaned duodenoscopes that were not in clinical circulation. Sampling may be more inefficient if performed by nonmicrobiologically trained individuals, such as endoscopy technicians in an endoscopy department. It is imperative that any individual who is sampling a duodenoscope for culture be trained in proper techniques. Finally, because the duodenoscopes used for our study were infrequently used clinically, they may lack potential biofilm. The lack of biofilm may not fully simulate conditions of duodenoscopes in actual use, as incomplete removal of biofilm would create conditions for bacterial to thrive despite high-level disinfection. Soiling of scopes used in routine clinical practice, or longer ATS-M inoculum drying times, could be used in future studies to address this limitation. We elected to use loaned scopes for this study, as the instruments in clinical use were not routinely available for our experiments.

In conclusion, agencies such as the FDA and American Society for Gastrointestinal Endoscopy have recommended that implementation of microbial culturing can supplement a strict, robust adherence to duodenoscope reprocessing in order to help reduce the risk of infection transmission by duodenoscope (14, 26). Based on our results, a microbial surveillance program utilizing the MEP would recover organisms from contaminated duodenoscopes at an approximate 65% recovery rate with a limit of detection around 10 CFU/scope. While this surveillance can detect reprocessing failures, it is important to note that, with only a 65% recovery rate, certain scopes may still be contaminated below the limit of detection. Thus, duodenoscope cultures should not be the sole basis when assessing risk of post-ERCP infections. Improved recovery could be tested by including neutralizer in the saline flush or by increasing the saline flush to 10 to 20 ml. Due to limitations of microbial sampling, risk assessment should also include active

![Visualization of sampling coverage on the distal end of a duodenoscope. (A) Distal end of the duodenoscope was coated in GlitterBug and viewed in ambient light (no UV), under UV light (before sampling), and then after sampling (after sampling) with either a flocked swab (top panel) or Olympus brush (bottom panel). (B) Visual comparison of the brush and flocked swab in ambient light (top panel) and then again after sampling under UV light to observe collected GlitterBug (bottom panel). UV, ultraviolet.](http://jcm.asm.org/)

**FIG 3** Visualization of sampling coverage on the distal end of a duodenoscope. (A) Distal end of the duodenoscope was coated in GlitterBug and viewed in ambient light (no UV), under UV light (before sampling), and then after sampling (after sampling) with either a flocked swab (top panel) or Olympus brush (bottom panel). (B) Visual comparison of the brush and flocked swab in ambient light (top panel) and then again after sampling under UV light to observe collected GlitterBug (bottom panel). UV, ultraviolet.
clinical surveillance of patients undergoing these procedures to identify the likelihood that a duodenoscope will become colonized with a high-concern organism. Assessment of certain patient factors, such as recent clinical culture results collected preprocedure, in addition to postprocedure clinical monitoring could serve as additional methods to more rapidly identify high risk situations. Periodic culture surveillance of duodenoscopes and reprocessing training can serve as routine tests for breakdown in cleaning procedures and ongoing quality indicators on processing these complex devices.

ACKNOWLEDGMENTS

We thank Preston Dahlgren and the dedicated endoscopy technicians at the LDS Hospital for loaning us the duodenoscopes and reprocessing the instruments after each experiment.

FUNDING INFORMATION

This study was funded by the Intermountain Healthcare Department of Clinical Epidemiology and Infectious Diseases and the Intermountain Healthcare Surgical Services Clinical Program. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

REFERENCES