REVIEW ARTICLE

Impact of wet storage and other factors on biofilm formation and contamination of patient-ready endoscopes: a narrative review

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The 2019 U.S. Food and Drug Administration report indicates that the clinical studies undertaken by the 3 main GI endoscope manufacturers demonstrate 5.4% of patient-ready duodenoscopes remain culture positive for high-concern organisms. The root causes of this persistent contamination are poorly understood. The objectives of this review include summarizing (1) the impact of inadequate manual cleaning and inadequate drying during storage on the formation of build-up biofilm in endoscope channels, (2) the impact of defoaming agents used during patient procedures on drying efficacy, (3) the data showing the importance of build-up biofilm on persistent microbial survival, and (4) the potential impact of implementation of a quality systems approach in GI endoscopy reprocessing. (Gastrointest Endosc 2020;91:236-47.)

INTRODUCTION

Endoscopic procedures have become some of the most commonly performed medical procedures. They are used to diagnose and treat a range of problems in the human GI tract. GI procedures using endoscopes are becoming increasingly more complex and invasive[1] and are being performed in sicker patients, many of whom are unable to withstand further deterioration. Concomitantly, there is an increasing recognition of residual microbial contamination of reprocessed patient-ready endoscopes. Reprocessing of flexible GI endoscopes has come under intense scrutiny because of multi-drug–resistant organisms (MDROs) transmitted by contaminated endoscopes that have caused infectious outbreaks.[2-8] The 9 clinical infections reported by Epstein et al[5] were culture positive for the New Delhi metallo-beta-lactamase (NDM) *Escherichia coli* outbreak strain in 13 clinical samples, including urine (3), abscess (2), blood (2), catheter tip (2), sputum (2), and wound (13). The identical NDM *E coli* strain was found to have contaminated the duodenoscopes used on these patients. The carbapenem-resistant Enterobacteriaceae (CRE) *Klebsiella pneumoniae* outbreak described by Humphries et al[8] did not indicate the types of

Abbreviations: AER, automated endoscope reprocessor; BBF, build-up biofilm; CDC-HICPAC, Centers for Disease Control-Healthcare Infection Control Practices Advisory Committee; CFU, colony-forming unit; CRE, carbapenem-resistant Enterobacteriaceae; FDA, U.S. Food and Drug Administration; HEPA, high-efficiency particulate air; HLD, high-level disinfection; MDRO, multi-drug resistant organism; MIFU, manufacturer's instructions for use; PAA, peracetic acid; QS, quality system; SEM, scanning electron microscopy; TB, traditional biofilm; VBNC, viable but nonculturable.

DISCLOSURE: *Dr Alfa has been involved as a consultant and in research projects for Olympus, KARL STORZ, 3M, Novaflux, and STERIS; has received royalties from HealthMark Industries; has participated as an invited conference speaker for Olympus, 3M, and Sealed Air, and as a speaker for a webinar for Ambu. Dr Singh has served on advisory boards for Pendopharm, Ferring Canada, Merck Canada, Takeda, Guardant Health; has received an educational grant from Ferring Canada; and a research grant from Merck Canada.*

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infections for the 9 patients involved in the outbreak, but they did indicate that the source patient had a positive urine culture with the outbreak strain. Furthermore, they reported that of the 6 patients infected with the CRE *K pneumonia* strain who died within 1 year of the outbreak, 2 deaths were attributable to the outbreak strain. Humphries et al[8] cautioned that "Reports of outbreaks due to CRE probably predominate largely because these organisms are flagged for additional investigation by hospital laboratories. However, clusters due to susceptible bacteria may be missed." They also recommended that improved surveillance after ERCP procedures is needed and that review of positive blood cultures after ERCP or active surveillance may improve detection of infections transmitted from contaminated duodenoscopes.[8]

McCafferty et al[9] recently reviewed PubMed, ScienceDirect, and CINAHL for publications describing infections and outbreaks from 2008 to 2018 that were related to contaminated GI endoscopes. The contributing factors and the outcomes of the outbreaks were assessed. They identified 16 publications that described duodenoscope-associated infections, and 2 that described colonoscope and gastroscope infections. There were outbreaks in the Netherlands, United States, United Kingdom, France, China, and Germany. The causative organisms included *Pseudomonas aeruginosa, E coli, K pneumoniae*, and *Salmonella enteritidis*. Contributing factors included lapses in reprocessing, biofilm formation, endoscope design issues, and endoscope damage. The authors indicated that improving endoscope reprocessing, detection of endoscope damage, and screening for contamination may prevent colonization and infection with MDROs and/or transmission of sensitive organisms by contaminated endoscopes. The retrospective study by Wang et al[10] provided the first insight into the risk of any type of infection after endoscopy. They reviewed all-payer claims data from 6 states in the United States and reported infection rates that occurred 7 days after endoscopy procedures for colonoscopy, osophagogastroduodenoscopy, and bronchoscopy in ambulatory surgery centers in the United States (they did not include data related to ERCP procedures). They found significantly higher 7-day postprocedure infection rates for screening colonoscopy (1.1 cases per 1000 procedures), nonscreening colonoscopy (1.6 cases per 1000 procedures), osophagogastroduodenoscopy (3.0 cases per 1000 procedures), and bronchoscopy (16.6 cases per 1000 procedures) compared with screening mammography (0.6 cases per 1000 procedures). The most-common types of infection involved the respiratory tract (eg, pneumonia), septicemia, and the GI system. The most-common organisms causing infection included *E coli, K pneumoniae, Clostridium difficile, Staphylococcus* spp, and unidentified Gram-negative bacteria. They concluded that postendoscopy infection rates were higher than previously thought. The Wang[10] study was limited, because it did not differentiate endogenous infections (ie, infections arising from the patient's own microorganisms) from exogenous infections with organisms originating from contaminated endoscopes.

The published clinical data clearly indicate that endoscopy procedures are not without risk and that transmission of infectious organisms (both MDROs and sensitive organisms) from contaminated endoscopes is a problem that needs to be addressed.

Transmission to patients from a contaminated endoscope can cause infection and/or colonization. The rectal screening data reported by Epstein et al[5] demonstrated that of 89 asymptomatic patients exposed to contaminated duodenoscopes, 27 (30%) became carriers. There were 8 infections directly linked to contaminated endoscope exposure and 27 carriers detected indicating a 3.4 times higher rate of transmission resulting in carriage versus infection. Data from some of the outbreaks have shown that once exposed to MDROs via a contaminated endoscope, the GI tract of patients can remain colonized for many months.[5] Zimmerman et al[11] reported that duration of carriage varied depending on whether the organism was initially detected in a diagnostic clinical specimen from a symptomatic patient (mean duration of carriage of 641 days) or the organism was detected from a rectal swab taken as part of surveillance culture from asymptomatic patients (mean duration of carriage 337 days). In addition, a recent review indicated that the 30-day mortality rates for blood stream infections with CRE, one of the MDROs, was 65.4% compared with 6.4% for non-CRE strains.[12] Even nonsystemic infections with CRE strains showed higher readmission rates and higher 90-day mortality rates.[13] The combination of long-term persistence of the carrier state and markedly adverse outcomes if there is a clinical infection has important implications. Even if the GI tract is only colonized with an MDRO from a contaminated endoscope, this still poses a long-term risk because subsequent patient exposure to antibiotics that kill the normal GI microbiome would allow the MDRO to replicate and potentially cause invasive infection, which can have much worse outcomes than with non-MDRO strains.

Contamination of patient-ready GI endoscopes linked to infectious outbreaks of MDROs led to a directive from the U.S. Food and Drug Administration (FDA) in 2015[14] to the 3 main GI endoscope manufacturers (ie, Olympus, Pentax, Fujinon) to conduct postmarket surveillance studies. The objective as stated by Shuren in the FDA statement (April 12, 2019)[15] was "Specifically, as part of their approved study plans, all 3 manufacturers are required to conduct a study to sample and culture reprocessed duodenoscopes that are in clinical use to learn more about issues that contribute to contamination, as well as a human factors study to assess whether trained hospital staff are following reprocessing instructions." The April 12, 2019 FDA interim report on the clinical studies undertaken by the 3 main GI endoscope manufacturers has

demonstrated that 5.4% of patient-ready duodenoscopes remain culture positive for high-concern organisms.

A key driver of persistent endoscope contamination is biofilm or build-up biofilm (BBF) formation during storage after full reprocessing, which includes cleaning and rinsing, high-level disinfection (HLD), alcohol flushing, and forced air drying.[16-23] Biofilm should not form inside dry, disinfected endoscope channels. However, if channels are wet during storage, then biofilm could form. The importance of drying endoscope channels is clearly stated in the manufacturer's instructions for use (MIFU) for all types of GI endoscopes. The following statement from the Olympus duodenoscope user manual[24] is an example of MIFUs regarding endoscope drying: "All equipment must be thoroughly dried prior to storage. Microorganisms proliferate in wet/moist environments." Furthermore, endoscope reprocessing guidelines from many countries reiterate the significance of drying reprocessed endoscopes for storage.[25-35] In some of these guidance documents, an alcohol flush of each channel is recommended to facilitate the drying process. Endoscope channel drying recommendations have been in place for many years. Despite these powerful cautionary statements, endoscope reprocessing personnel are unable to define "How dry is dry enough?" and moisture has been reported to persist in stored endoscope channels, facilitating microbial replication.[22,36,37]

The objective of this review is to provide the current data on the extent of moisture persistence in endoscope channels, the impact of simethicone (a defoaming agent) on drying efficacy, the impact of moisture and the potential impact of simethicone on biofilm and BBF formation, and most importantly, the urgent need for quality system (QS) audits in endoscopy clinics.

DRYING OF ENDOSCOPE CHANNELS DURING STORAGE

The importance of drying during endoscope storage was recognized as early as 1983 when the use of glutaraldehyde instead of alcohol was introduced for endoscope reprocessing. Gerding et al[38] stated the following: "Institution of forced air drying significantly reduced bacterial contamination of stored endoscopes, presumably by removing the wet environment favorable for bacterial growth." Alfa and Sitter's[17] study was the first to conclusively demonstrate that if reprocessed duodenoscopes were stored with moisture in the channels, significant bacterial proliferation would occur in the instrument channel during storage over 24 to 72 hours. This proliferation could be eliminated when 10 minutes of forced air drying was used (note that this drying was without an alcohol flush before drying). The importance of thorough drying of endoscope channels has also been reflected for many years in the endoscope MIFUs as well as in guidance documents. The problem is that neither the MIFUs nor the guidance documents define what level of dryness is required, and furthermore, they do not specify how health care personnel can audit endoscope channels to determine whether the required level of dryness has been achieved. For example, the current Olympus instructions for duodenoscopes (2015)[24] states: "All equipment must be thoroughly dried prior to storage. Microorganisms proliferate in wet/moist environments." The Society of Gastroenterology Nurses and Associates (SGNA)[33] and the French[39] guidelines both emphasize the importance of drying but inadvertently indicate that forced air drying (with or without an alcohol flush) could be achieved "… (either by AER or manually)." Thus, it is not surprising that reprocessing personnel often believe that the use of automated endoscope reprocessors (AERs) that provide an alcohol flush and forced air drying (often the default dry time is 1 minute) as part of their cycle ensures they are meeting the MIFU requirement for dry storage. AER manufacturers do not indicate that the alcohol flush and air flush in their AERs is sufficient to ensure dry storage.

The recent utilization of borescopes to visualize the internal channels of flexible endoscopes has demonstrated that residual fluid in endoscope channels is widespread despite AER processing followed by overnight storage in regular storage cabinets.[21,22,36,40,41] Ofstead et al's[40] clinical study was the first to show the value of using borescope assessment to determine if there was channel damage, organic residues, or moisture in GI endoscope channels after full reprocessing and overnight storage. Ofstead et al's[36] clinical study at one ambulatory endoscopy clinic reported that on borescope examination, 95% of the GI endoscopes evaluated had residual fluid detected after full AER cycles (including an alcohol flush and 6 minutes of air flushing) and overnight storage in a closed ventilated endoscope storage cabinet (this type of cabinet does not flush air through the channels during storage). Subsequently, they performed a clinical study in 3 multispecialty hospitals that were part of a Joint Commission accredited large health care system and reported by borescope and humidicator paper testing that 49% of the GI endoscopes had residual fluid in their channels.[41] The presence of fluid varied greatly by facility; 1 of the 3 sites had no detectable fluid in endoscopes after overnight storage, whereas the other 2 sites had 85% of endoscopes with residual fluid. The site with no detectable fluid had implemented 10 minutes of manual forced air flushing of endoscope channels before storage, whereas the other 2 sites relied on the AER alcohol flush and air purge cycle and did not perform any additional drying before storage. A key component of this study was data demonstrating that humidicator test strips could be used as an alternative to borescope examination to detect residual fluid, because they had 95.5% positive predictive value and 100% negative predictive value.[41] These test strips changed from a dark blue color to a pink color when even as little as 10 μL of fluid was placed on the humidicator strips.[41]

TABLE 1. Borescope assessment of the impact of various channel drying methods on residual fluid droplets in endoscope instrument channels

Hours stored in endoscope storage cabinet*	AER with alcohol flush and 1 to 6 minutes air flush (%)†	AER with alcohol flush and 1 minute air flush plus manual forced air flushing for 10 minutes (%)‡	AER with alcohol flush and 1 minute air flush plus manual forced air drying for 10 minutes§	AER with alcohol flush an 1 minute air flush plus automated drying for 5 minutes§	AER with alcohol flush and 1 minute air flush plus automated drying for 10 minutes§
Before placed in storage cabinet			4.55 (6.14)	0.83 (1.29)	0 (0)
24 hours	42-95	Not done	1.62 (1.61)	0.20 (0.34)	0.01 (0.07)
48 hours	Not done	42.6	0.51 (0.7)	0.04 (0.11)	0 (0)
72 hours	Not done	Not done	0 (0)	0 (0)	0 (0)

AER, Automated endoscope reprocessor.
*The endoscope storage cabinets used did not flush air through the channels during storage (ie, they were not channel-purge storage cabinets).
†Data extracted from Ofstead et al.[36,41] Results are reported as the percentage of endoscopes with fluid droplets visible on borescope examination of the instrument channel.
‡Data extracted from Barakat et al.[22] Results are reported as the percentage of endoscopes with fluid droplets visible on borescope examination of the instrument channel.
§Data extracted from Barakat et al.[22,23] Results reported as the average number of fluid droplets visible on borescope examination of the instrument channel. The manual method consisted of using pressurized medical-grade air and a trigger-nozzle to manually flush the channels. The automated method was a DryScope flushing pump that had an adjustable timer to control the duration of flushing channels with high-efficiency particulate air.

Similarly, Perumpail et al[37] reported that cobalt chloride test paper changes color when as little as 5 μL of water is inoculated directly on the test strip. Perumpail et al[37] inoculated various amounts of water into duodenoscope channels and determined that the limit of detection for water is 250 μL, 100 μL, and 50 μL for the air-water, suction biopsy, and elevator channels, respectively.

The findings on residual fluid reported by Ofstead et al[41] were further supported by Barakat et al,[22] who did a clinical study in an academic center that performed more than 50 GI endoscopy procedures per day. Barakat et al[22] found that 42.6% of GI endoscopes had residual fluid despite a full AER cycle (including alcohol flush and air drying) as well as 10 minutes of manual forced air drying. They found that colonoscopes were significantly more likely to have residual fluid compared with duodenoscopes and that fluid was more likely to be detected the shorter the duration duodenoscopes were in the endoscope storage cabinet. Table 1 summarizes the data from Ofstead et al[36,41] and Barakat et al[22] clearly documenting that fluid within GI endoscope channels is difficult to eliminate and convincingly demonstrates that AER cycles with alcohol and air flushes are not adequate to meet the endoscope MIFUs for drying before endoscopes are stored in regular storage cabinets. The extent of lack of adherence to measures to prevent wet storage in the United States is reflected by Thaker et al's[42] survey data showing that less than half the 249 sites surveyed in the United States reported the use of manual forced air drying before storage of duodenoscopes. As stated by Thaker et al,[42] it is "alarming" that there is such poor compliance with the multisociety guideline[35] that recommends thorough GI endoscope drying before storage.

There are few published data to support the use of an alcohol flush before drying of endoscopes for storage. Barakat et al[22] discussed the role of the alcohol flush in the drying process and indicated that the British guidelines do not recommend an alcohol flush,[34] because this could act as a fixative for any residual protein material. Indeed, Barakat et al's[22] data indicated that effective drying was achieved by the 10-minute automated drying method without a preceding manual alcohol flush. In this study, there was an alcohol flush followed by 1 minute of air flushing in the AER, but it is unlikely that sufficient alcohol would remain in the channel and contribute to the subsequent drying provided by manual or automated methods. Alfa and Sitter[17] reported effective drying of duodenoscope channels using forced air drying without the use of alcohol. Furthermore, the simulated-use study by Singh et al[43] showed that the use of an alcohol flush versus no alcohol flush did not improve endoscope contamination rates when reprocessed duodenoscopes were stored in channel-purge storage cabinets.

Barakat et al[22] compared different methods of drying of the GI endoscope working channels and found that manual forced air flushing (medical-grade air) for 10 minutes was not as effective as automated high-efficiency particulate air (HEPA)-filtered air flushing for 10 minutes (4.55 versus 0 fluid drops, respectively). There were rare fluid drops detected after 24 hours of storage despite no drops detected immediately after the 10 minutes of automated air flushing. This may represent trace amounts of moisture remaining in the channel that only coalesce to form droplets after a period of storage. These data suggest that more than 10 minutes of automated air flushing may be needed to thoroughly dry endoscope channels before storage. A more effective long-term strategy than 10 minutes of automated air flushing would be to place the endoscope in a channel-purge storage cabinet that provides more extensive air flushing as recommended by the British Society of Gastroenterology.[34] The requirement for channel-purge storage cabinets for all types of endoscopic instruments (other than those stored packaged after sterilization) has recently been mandated in Australia[44] with a phase-in period of 3 years. Use of a channel-purge storage

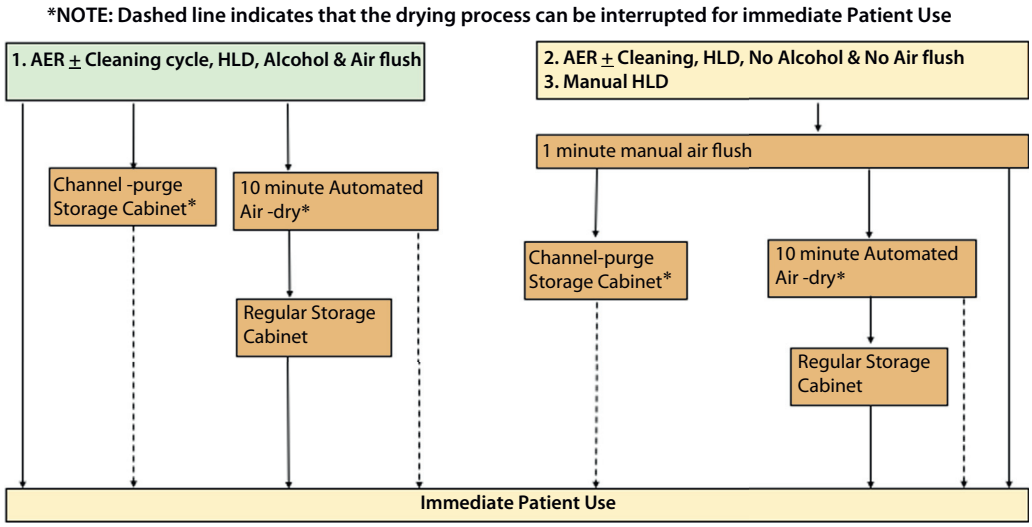

Figure 1. Logistical workflow for drying flexible endoscopes.

cabinet provides "engineered safety," because it ensures dryness of endoscope channels during storage and is less influenced by human factors. This recommendation is supported by Perumpail et al's[37] report demonstrating that without an alcohol flush of the endoscope channels, a channel-purge storage cabinet was able to dry the internal channels of bronchoscopes, duodenoscopes, and colonoscopes within 1 hour, whereas the standard storage cabinet (no airflow through the endoscope channels or the cabinet) was not able to achieve internal channel drying even after 24 hours. Furthermore, they demonstrated that there was substantial replication of *P aeruginosa* from 3.5 $\log_{10}$ colony-forming units (CFUs) (inoculum) up to 7 to 9 $\log_{10}$CFU by 24 hours storage in the standard cabinet. When inoculated endoscopes were stored in the channel-purge storage cabinet, the viable count dropped to <1 $\log_{10}$CFU of *P aeruginosa* within 3 hours and remained at about this level up to 31 days[37] for all endoscope types evaluated. These data clearly demonstrate that prolonged moisture in endoscope channels results in multi-log microbial replication, which is the initial stage of biofilm formation; this is a setting of residual moisture but little shear and no nutrient exchange.[45]

To ensure that extraneous materials are not introduced, the quality of air used for drying endoscope channels is an important consideration. For medical device reprocessing, Chobin[46] has recently recommended the use of "instrument-grade" air (previously called "medical-grade compressed air") that has undergone filtration to 0.01 μm. Instrument-grade air is normally used for pneumatic equipment valves and electrical controls. Instrument-grade air (or medical-grade air) is provided in pressurized tanks or using compressed air that meets specified purity in terms of particles, humidity, and oil. HEPA filtration achieves 0.03 μm filtration (particles and microbes) and is a "point-of-use" filtration method for ambient air. HEPA filtration is the process used for biosafety containment cabinets to produce particle-free, microbe-free air and is the process used to remove the risk of air-borne pathogens such as *Mycobacterium tuberculosis*, Ebola virus, and others exhausted from negative pressure rooms in health care facilities. Furthermore, it has been shown to be an acceptable grade of high-quality air for drying flexible endoscope channels.[22,34,47,48] Thus, instrument-grade air or HEPA filtration is appropriate for drying flexible endoscope channels using automated flushing pumps or channel-purge storage cabinets. The low humidity in instrument-grade air versus room air that is HEPA filtered should facilitate faster drying, but there are no published data to demonstrate that this difference is critical for endoscope drying because Barakat et al's[22] data indicated that after 10 minutes of drying using an automated pump and HEPA-filtered room air, there was no residual moisture detected by borescope examination or by humidicator strips.

A question that frequently comes up but is not addressed in current guidelines is this: How much drying is needed for fully reprocessed endoscopes between successive patient procedures? Logically, if an endoscope is fully cleaned and disinfected by manual or automated methods, it is not truly necessary to totally dry the endoscope channels between patient procedures performed at short intervals. However, the logistical problem is that the reprocessing staff may not know if the endoscope will be used immediately or not. Functionally, it is inappropriate to have an endoscope "dripping" wet during transport for the next patient procedure. A logical approach to this dilemma is outlined in Figure 1. This approach is based on whether the endoscopy site uses an AER that has an air flush cycle (± alcohol flush as part of the cycle) or uses manual HLD. In each case, the endoscope should undergo at least 1 minute of air flushing before use on

the next patient, thereby ensuring it is not dripping wet when transported to the procedure room. In addition, Figure 1 outlines the use of either regular endoscope storage cabinets or channel-purge storage cabinets.

THE IMPACT OF SIMETHICONE ON DRYING EFFICACY

Simethicone is a defoaming agent used to eliminate bubbles on the GI mucosal surface to improve visualization of abnormalities.[49] Simethicone has been used in many types of flexible endoscopes, and historically it was used in the water bottle for colonoscopy and gastroscopy and less frequently in duodenoscopy or other GI endoscopic procedures. The exact extent of simethicone usage in GI endoscopy is unknown but appears to be extensive.[50] As early as 2009, Olympus warned of the difficulty of MIFU cleaning being able to remove high concentrations of simethicone and recommended using the lowest possible concentration to achieve the desired clinical effect. Currently, all 3 major GI endoscope manufacturers do not support the use of simethicone, because it is insoluble in alcohol and water and is not easily removed by the current MIFUs for endoscope cleaning. On the other hand, there are distinct clinical benefits of simethicone use. Kutyla et al[49] reported that simethicone added to the water bottle during colonoscopy resulted in a 100% increase in polyp detection compared with water alone. Alternatively, clinical studies have provided data to support the use of simethicone in the bowel preparation stage or taken by the patient 20 minutes before endoscopy instead of in the water bottle,[51,52] because these approaches provided similar improvements to mucosal visibility without the need to flush simethicone through the endoscope water channel. However, suctioning during the patient procedure will draw ingested simethicone through the suction channel, and whether this detrimentally affects the scope channels remains unknown. An ECRI Alert[53] outlined that Olympus has recommended that if endoscopy sites decide to use simethicone for clinical reasons, it should be used at the lowest concentration and be injected through the instrument channel. They also suggest that 2 rounds of manual cleaning may be helpful. This approach is also reflected in the Canadian Association of Gastroenterology position statement.[54] The recent position statement of the Gastroenterological Society of Australia[44] has concluded "… we believe that continued use of simethicone is appropriate, and it can be administered through any endoscope channel." A relevant question is this: Does the use of simethicone injected through the endoscope water channel or the instrument channel or with oral ingestion have any detrimental effect on endoscope reprocessing?

Barakat et al[23] evaluated the impact of simethicone concentration on residual fluid in endoscope channels and reported that the use of 1% and 3% simethicone injected through the working channel resulted in significantly more fluid droplets (13.5 and 17.3 droplets, respectively) after full reprocessing compared with when only water or 0.5% simethicone was used (6.3 and 5.8 droplets, respectively). The use of 1% to 3% simethicone did result in an increased number of fluid droplets within the fully reprocessed GI endoscopes despite full AER processing combined with 10 minutes of manual forced air flushing. Although the number of residual droplets after use of 0.5% simethicone was no different compared with use of water alone, the retained droplets contained simethicone after full reprocessing.

Clearly, there is an urgent need for novel cleaning technologies that can effectively remove non-water soluble defoaming or lubricating agents used in endoscopy procedures. An alternative solution would be development of new defoaming and lubricating agents that can be effectively removed by existing endoscope cleaning protocols. More research is urgently needed to provide effective solutions to this problem.

CONTAMINATION OF ENDOSCOPES: TRADITIONAL AND BUILD-UP BIOFILMS

Although there are multiple factors that contribute to contamination of flexible endoscope channels, a major one is the formation of traditional biofilm (TB) and/or BBF. Both TB and BBF have been shown to reduce the efficacy of both HLD and liquid chemical sterilization.[19,55,56] TB forms during continuous hydration and theoretically should not form within properly reprocessed endoscope channels that are stored totally dry. Alfa and Sitter[17] clearly demonstrated that inadequate drying of reprocessed patient-used duodenoscope channels led to high levels of bacterial replication during storage. BBF develops over repeated rounds of exposure to fluids (patient mucosa, bedside flush, transport, cleaning, liquid chemical disinfection/sterilization, rinsing) and dry storage.[57] BBF forms gradually with successive rounds of reprocessing (like the concentric layers of an onion) and is more compact and adherent than TB.[57] BBF formation is relevant to flexible endoscope channels and encompasses aspects of partial TB formation along with partial fixation and nonsterile storage. BBF formation and rebound of viable but nonculturable (VBNC) bacteria within BBF is accelerated under wet storage conditions.[58] As outlined in the preceding section, achieving truly dry storage of endoscope channels has been an elusive goal in the clinical setting. The study by Alfa et al[19] demonstrated that the current MIFUs for cleaning may not completely remove TB in endoscope channels, and these TB residuals will eventually form BBF, which protects embedded bacteria from the killing effect of HLDs. As far back as 2004[16] and as recently as 2014,[18] scanning electron microscopy (SEM) studies have shown the

presence of BBF-like accumulations within patient-used repeatedly reprocessed endoscope channels. These SEM studies as well as the recent borescope studies showing fluid and organic material residues (similar in appearance to BBF residues described by Alfa et al[19]) within endoscope channels[41] provide evidence that the lack of inspection of the inside of endoscope channels has resulted in a false sense of security regarding endoscope reprocessing (ie, erroneous belief that the MIFUs for adequate drying during storage are being achieved by the AER of manual flushing of air in the channels).

It is clear from the recent publications that contamination of patient-ready endoscopes is still an ongoing problem with ≥1 CFU detection rates on culture ranging from 18.3%[59] to 65%.[60] Rauwer et al's[60] study showed that there was no significant difference in the contamination rates reported by 26 health care centers in the Netherlands. Furthermore, they demonstrated that 15% of the duodenoscopes tested contained organisms of concern (ie, derived from the GI or oral tract of humans). Their conclusion was, "The observed nationwide high prevalence of contamination of patient-ready duodenoscopes is a clear indication that the current combination of reprocessing and process control is not sufficient."

The preliminary FDA report (April 12, 2019) of the post-market clinical studies[15] indicated that the expected <0.4% contamination rate in patient-ready duodenoscopes was not achieved. They reported[15] that in properly collected instrument channel samples[61] from fully reprocessed duodenoscopes, 3.6% had microbes of low to moderate concern and an additional 5.4% had ≥1 CFU of organisms of concern. The total 9% rate of "actionable" cultures in the FDA clinical studies in the United States is lower than the 15% rate in the Netherlands,[9] but does further emphasize that contamination in patient-ready duodenoscope channels is an ongoing worldwide problem.

There is a need to identify and eliminate the factors that contribute to development of BBF within endoscope channels. The recently developed BBF model[56] provides a worst-case challenge for assessing endoscope channel cleaning protocols and for determining the efficacy of disinfection/sterilization methods. The primary significance of BBF in endoscope channels is that microbes embedded within BBF can persistently resist HLD.[56] The microbes in BBF enter a VBNC state as described by Li and Ye[12] but on storage can return to a culturable state as documented for *P aeruginosa* by Alfa et al.[56] This may explain why cultures of endoscopes are often negative, and contaminating organisms are not detected when samples are collected immediately after HLD and without neutralizer.

It is also important to recognize the role of environmental (low-concern) bacteria in persistent endoscope channel contamination. Many of the recent clinical culture studies have documented the widespread presence of *Bacillus* species in endoscope channels.[48,60,62] This type of organism is not an unexpected contaminant, because it is a ubiquitous, environmental spore-forming organism that would not be eliminated by HLD. It is frequently ignored in endoscope culture results when at low levels (<10 CFU), because it is considered not clinically significant. However, the recent study by Johani et al[20] reported that all 39 endoscope channels evaluated contained biofilm, and the top 3 organisms detected by molecular sequencing were *Methylobacterium* (35%), *Ralsonia* (12%), and *Bacillus* (9%), all environmental organisms. These data support Singh et al's[43] mock clinical study in which they reported that full manual cleaning, disinfection in an AER, and channel-purge storage ensured there was no detectable *E coli* in any of the 119 repeated duodenoscope challenges (ie, duodenoscope channels were inoculated with high organic load and high levels of *E coli* and *Enterococcus faecalis* for 2 hours and then fully reprocessed following the MIFU) after storage for 1 to 5 days. However, there were environmental organisms detected, and the most-common environmental isolate from fully reprocessed duodenoscopes was *Bacillus* species. This organism was also found in the endoscope storage cabinet.[43] The study by Bridier et al[55] raises serious questions about the importance of environmental organisms within endoscope channels, not as potential human pathogens but as biofilm matrix formers that provide "by-stander" protection to pathogenic organisms that become embedded in the existing TB or BBF matrix. Bridier et al[55] demonstrated this capability using a *Bacillus subtilis* strain that was isolated from an AER and was resistant to peracetic acid (PAA) in its biofilm form. The data confirmed that when *Staphylococcus aureus* (susceptible to PAA in mono-microbic biofilm) was embedded within the *B subtilis* biofilm matrix, it was protected from killing by PAA. This raises questions about a similar symbiotic relationship in patient-used flexible endoscopes whereby environmental organisms replicate under moist conditions, creating a persistent BBF matrix. During patient procedures, human pathogens can incorporate into the environmental matrix and thereby may gain "by-stander" protection from HLDs (and potentially from low-temperature sterilization). The study by Johani et al[20] confirmed that VBNC organisms were detectable within a biofilm matrix in fully reprocessed patient-used endoscope channels. The "by-stander" protection described by Bridier et al,[55] combined with VBNC state described by Johani et al,[20] may explain why some endoscope channels have false-negative culture results, especially if samples are collected immediately after HLD.

THE IMPACT OF MOISTURE AND SIMETHICONE ON BBF FORMATION

There is no doubt that residual moisture stimulates bacterial replication and can lead to biofilm

TABLE 2. Current endoscope reprocessing recommendations from guidelines

Guideline*	Simethicone for patient procedure	Manual or AER cleaning and rinsing	Monitoring of manual cleaning	HLD or sterilization	Alcohol flushing and drying	Storage	Monitoring of residual moisture	Culture of endoscopes and AER
Canada†								
PIDAC, 2016[25]	N/A	Manual ± AER‡	Yes	Either, GI scopes HLD	Yes	Regular cabinet	No	Outbreak only
Health Canada, 2010[26]	N/A	Manual or AER‡	Yes	Either, GI scopes HLD	Yes	Regular or channel-purge cabinet	No	No
USA								
SGNA, 2015[33]	N/A	Manual ± AER‡	N/A	Either, GI scopes HLD	Yes§	Regular cabinet	No	No
AAMI ST91, 2015[28]	N/A	Manual + AER‡	Yes	Either, GI scopes HLD	Yes§	Regular cabinet or channel-purge cabinet	No	No
Multisociety; Petersen et al, 2017[35]	N/A	Manual + AER‡	N/A	Either, GI scopes HLD	Yes	Regular cabinet	No	No
ASGE: Calderwood et al, 2018[68]	N/A	Manual	N/A	Either, GI scopes HLD	Yes	Regular cabinet	No	No
Australia								
GESA Consensus Endoscopy Guideline, 2019[44]	N/A	Manual ± AER‡	N/A	AER required for HLD	Yes	Channel-purge cabinet required	No	Yes
GENCA/GESA, 2010[30]	N/A	Manual ± AER‡	N/A	Either	Yes	Channel-purge or regular cabinet	No	Yes
Europe								
ESGE/ESGNA: Beilenhoff et al, 2017[31]	N/A	Manual ± AER‡	Suggested	GI scopes HLD	Flush dry, no alcohol	Channel-purge or regular cabinet	No	Yes
Saviuc et al, 2015 (France)[39]	N/A	Manual ± AER	N/A	HLD for semi-critical, sterilization for critical scopes	Flush dry, no alcohol§	Regular storage cabinet	No	Yes
BSG, 2016 (UK)[34]	N/A	Manual + AER‡	N/A	AER required for HLD, sterilization for critical scopes	Flush dry, no alcohol	Channel-purge recommended	No	Yes

AAMI, Association for the Advancement of Medical Instrumentation; *AER*, Automated endoscope reprocessor; *ASGE,* American Society for Gastrointestinal Endoscopy; *BSG,* British Society of Gastroenterology; *ESGE,* European Society of Gastrointestinal Endoscopy; *ESGNA,* European Society of Gastroenterology Nurses and Associates; *GENCA,* Gastroenterological Nurses College of Australia; *GESA,* Gastroenterological Society of Australia; *PIDAC*, Provincial Infectious Diseases Advisory Committee; *SGNA,* Society of Gastroenterology Nurses and Associates; *HLD*, high-level disinfection; *N/A*, not addressed.

*All the guidelines listed recommend that audits be done as part of a quality system approach but often do not provide tools for such audits.

†ECRI 2019: Safety alert warning against use of simethicone. Sites can opt to use simethicone based on clinical importance.

‡Only AERs with validated cleaning cycles.

§Drying can be provided by manual flushing of air before storage or by AER with an air flush as part of the cycle.

formation.[17,37,43,45] However, this has been further complicated by the use of simethicone or other insoluble off-label lubricants and defoaming agents described by Ofstead et al.[50] Simethicone consists of silica particles and simethicone oil, which are both insoluble in alcohol and water and are not reliably removed by the current MIFUs for cleaning. In addition, simethicone suctioned through the instrument channel increases the amount of residual fluid droplets after full reprocessing.[23] The data on the impact of simethicone suggest that it may enhance BBF formation, because it prevents drying within endoscope channels[23] and may contain sugar and thickening agents that provide a source of nutrition[50,54] that would facilitate bacterial replication for any microbes present (ie, environmental microbes or pathogenic human organisms). Although studies have demonstrated simethicone residuals in patient-ready endoscopes,[23,50,56] no published study has yet provided data demonstrating that simethicone directly increases TB or BBF formation. It is possible that if additional measures are taken to improve the removal of

simethicone during cleaning and avoid moisture in endoscope channels during storage, TB or BBF may not occur with simethicone use. This is an important consideration in view of the benefits of simethicone use on detection of GI lesions as discussed above.

Table 2 summarizes what various endoscope reprocessing guidelines indicate about endoscope reprocessing steps, and it is clear that simethicone use has not been a consideration, which needs to change. It is also clear that drying endoscopes for storage is required by all guidelines, but the use of channel-purge storage cabinets is not prevalent in North America, whereas it is mandated in Australia. Automated HLD by AERs is mandated in England and Australia but not in other countries.

STERILIZATION OF ENDOSCOPES

If an endoscope is deemed "critical" because it enters a sterile body site or breaks the mucosal surface, it should be sterilized (eg, bronchoscopes, cystoscopes, ureteroscopes), whereas if the endoscope is "semi-critical" (eg, colonoscopes, gastroscopes, etc) it should receive minimal HLD. Most guidelines support the use of HLD for GI endoscopes of all types despite biopsies and other procedures that break the mucosal surface (Table 2). Recently, there has been a push to move toward sterilization for all flexible endoscopes.[63] This would address the issue of wet storage because all low-temperature sterilization cycles will fault if there is too much moisture in the load. Furthermore, it would provide sterile storage (providing endoscopes are kept inside the wrap they are sterilized in). Although sterilization has a wider margin of safety than HLD, it is important to recognize that if cleaning is not done properly, BBF will still accumulate, and failure of low-temperature sterilization to eradicate MDROs has already been shown to occur.[64]

QUALITY SYSTEM AUDITS FOR ENDOSCOPY

It is clear that flexible endoscope reprocessing is lacking in terms of a QS approach. As early as 2015, the Centers for Disease Control and Prevention (CDC)[65] sent out a Health Advisory entitled "Immediate need for healthcare facilities to review procedures for cleaning, disinfecting and sterilizing reusable medical devices," and in 2016, the Provincial Infectious Diseases Advisory Committee (PIDAC)[25] advised "If the health care facility has not recently conducted observational audits of endoscope reprocessing, and particularly duodenoscope reprocessing, it must conduct an audit immediately and then repeat audits regularly (annually at the minimum) or when practices change." Despite these alerts, the issues related to contaminated endoscopes have continued. In a recent clinical study, Rauwers et al[60] concluded "Additional preventive measures including microbial surveillance strategies are needed to reduce the number of contaminated duodenoscopes." The numerous clinical studies showing widespread wet storage, simethicone residuals, and endoscope channel contamination support the CDC-HICPAC (Centers for Disease Control-Healthcare Infection Control Practices Advisory Committee)[66] call for changes in endoscope reprocessing that better align with a QS approach. The Gap Analysis tool and Endoscope Reprocessing Audit tools provided by the CDC-HICPAC[66] are modifiable and are an excellent platform for endoscopy centers to use to identify gaps in endoscope reprocessing and implement changes to improve the overall process. Because the field is ever changing, it is important to update the CDC-HICPAC 2016[66] base audit documents as new parameters are identified. For example, the addition of questions related to use of lubricants, tissue glue, and defoaming agents, monitoring of manual cleaning, and monitoring of drying adequacy of endoscope channels need to be added to the existing document. Table 3 provides an overview of the essential steps that should be monitored for GI endoscopes, the data to collect, and the actions to be taken based on the monitoring data. For example, monitoring the manual cleaning step is essential to ensure that an adequate level of cleaning is consistently achieved (eg, ≥95% pass for cleaning monitoring)[67] and that the data are reviewed on an ongoing basis with each reprocessing staff person as part of competency assessment.

The corrective action taken (eg, re-training, enhanced monitoring) must be documented when endoscope manual cleaning adequacy does not achieve expected benchmarks. Similarly, monitoring drying adequacy is another essential step, because this is a key parameter that determines whether bacterial replication can occur during storage. If there is moisture detected in endoscope channels after overnight storage, the site manager, in discussion with infection prevention and control staff, may decide to perform endoscope cultures to assess the presence of contamination within endoscope channels as outlined in the FDA culture protocol.[61] The third key aspect is determining if defoaming agents (or other water-insoluble products) are used during the patient procedure, because these products can interfere with cleaning and drying of GI endoscopes. In addition to the overview in Table 3, sections in the CDC-HICPAC[66] documents address other important QS considerations, including administration, policies, documentation, inventory, physical setting, education, training, competencies, as well as disinfection/sterilization breaches or failures. A QS approach to all aspects of flexible endoscope reprocessing cannot be implemented overnight; continued incremental steps are required. For sites starting this process, the 3 key first steps to implement are rapid monitoring of manual cleaning compliance, implementation of automated endoscope channel drying, and monitoring of dry storage.

TABLE 3. Overview of essential steps related to endoscope reprocessing

Essential steps	Monitoring	Data analysis and action
Procedural (ie, aspects of patient procedures that affect reprocessing)	Determine if lubricants, defoaming agents, tissue glue, etc, are used for any patient procedure. Documentation of any endoscope problems (eg, blockage, accessory extraction problems, broken accessories, etc).	Document facility approval for all off-label substances used. Frequency of endoscope problems and documentation of response and corrective actions taken.
Pre-clean	Document completion for each scope; staff sign-off.	Percentage missed (ie, no staff sign-off). Documentation of corrective actions taken.
Prolonged time in transit (ie, >1 hour)	Document time that pre-clean is completed and time manual cleaning starts.	Document percentage with prolonged transit. Document that additional cleaning was performed when prolonged transit occurs. If due to after-hours emergency procedures, develop after-hours reprocessing staff plan.
Leak test	Document completion for each scope; staff sign-off.	Percentage missed (ie, no staff sign-off). Leak test failure rate (%). Documentation of corrective actions taken.
Manual clean	Rapid testing of cleaning compliance; eg, ATP or organic residuals (minimally; testing of some scopes each day). Document initial testing, re-cleaning, and re-test.	Percentage failure rate. Percentage failure after re-clean. Data review with reprocessing staff (weekly or monthly). Individual data should be part of ongoing documentation of staff competency.
Visual inspection	Document completion for each scope (define abnormalities to check; eg, oily or other deposits, epoxy integrity, lens integrity, etc); can use borescope for magnification during external visual inspection. Staff sign-off.	Percentage missed (ie, no staff sign-off). Percentage of faults detected. Document corrective actions taken. Return rates to manufacturer.
Disinfection/ sterilization	Test and document. Minimum effective concentration of disinfectant, biological indicator, chemical indicator as appropriate. AER; document sub-micrometer filter changes. Monthly culture of endoscope channels and/or final AER rinse water (if done at this endoscopy site).	Percentage of failures detected. AER; documentation of culture results and corrective actions taken if CFU exceeds limits (if done at this endoscopy site).
Drying/ storage	Document at least 10 minutes automated drying performed for each scope if external automated drying pump is used; staff sign-off. Document function of drying pump (external flushing pump or channel-purge storage cabinet) as per MIFU. Humidicator test or borescope test of scope channels after overnight storage (minimally test some scopes each week). Internal channel inspection by borescope; monthly for each scope (define actionable lesions). Culture of endoscope channels on periodic basis (if done at this endoscopy site).	Percentage missed drying (ie, no staff sign-off). Percentage failure of drying based on humidicator test or borescope inspection. Percentage abnormalities identified by borescope inspection; monthly summary of borescope abnormalities reviewed with reprocessing staff. Document corrective actions taken for any lapses detected in monitoring.
Documentation	All monitoring records summarized and reviewed. Sign-off by supervisor.	Review all monitoring data and corrective actions taken with reprocessing staff on a weekly basis and with infection control on a monthly basis. Include monitoring data in yearly competency review for each reprocessing staff person.

ATP, Adenosine triphosphate; AER, automated endoscope reprocessor; *CFU*, colony-forming unit; *MIFU*, manufacturer's instructions for use.

CONCLUSIONS

It is becoming increasingly apparent that there is an immediate need to focus attention on the under-recognized but widespread issue of moisture in endoscope channels during storage (including the potential role of simethicone and other off-label products in preventing cleaning and drying adequacy). Unless a QS approach is implemented, the accumulation of TB and BBF in endoscope channels will continue to result in contamination of flexible endoscopes that protects embedded microbes against HLD and low-temperature sterilization, which could result in infection transmission.

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