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Major article

Persistent contamination on colonoscopes and gastroscopes detected by biologic cultures and rapid indicators despite reprocessing performed in accordance with guidelines

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Background: Pathogens have been transmitted via flexible endoscopes that were reportedly reprocessed in accordance with guidelines.
Methods: Researchers observed reprocessing activities to ensure guideline compliance in a large gastrointestinal endoscopy unit. Contamination was assessed immediately after bedside cleaning, manual cleaning, high-level disinfection, and overnight storage via visual inspection, aerobic cultures, and tests for adenosine triphosphate (ATP), protein, carbohydrate, and hemoglobin.
Results: All colonoscopes and gastroscopes were reprocessed in accordance with guidelines during the study. Researchers collected and tested samples during 60 encounters with 15 endoscopes. Viable microbes were recovered from bedside-cleaned (92%), manually cleaned (46%), high-level disinfected (64%), and stored (9%) endoscopes. Rapid indicator tests detected contamination (protein, carbohydrate, hemoglobin, or ATP) above benchmarks on bedside-cleaned (100%), manually cleaned (92%), high-level disinfected (73%), and stored (82%) endoscopes. Visible residue was never observed on endoscopes, but it was often seen on materials used to sample endoscopes. Seven endoscopes underwent additional reprocessing in response to positive rapid indicators. Control endoscope channels were free of biologic residue and viable microbes.
Conclusion: Despite reprocessing in accordance with US guidelines, viable microbes and biologic debris persisted on clinically used gastrointestinal endoscopes, suggesting current reprocessing guidelines are not sufficient to ensure successful decontamination.

Gastrointestinal (GI) endoscopes are complex instruments that become highly contaminated during use. Endoscope reprocessing is a multistep process consisting of cleaning and high-level disinfection (HLD).[1] Manual (mechanical) removal of debris from external surfaces and interior channels is a fundamental step of reprocessing.[1-3] Residual substances not removed during cleaning may interfere with disinfectants.[3-5] Biofilm, an accumulation of biomass containing microbes and other material, adheres to surfaces, forms

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a matrix that may be difficult to remove during subsequent reprocessing,[5] and may prevent disinfectants from inactivating microbes.[5]

An accurate estimate of the infection risk associated with endoscopic procedures has yet to be determined.[6] Most infectious outbreaks linked to endoscopes have been associated with documented breaches of reprocessing.[7-9] Recently, outbreaks involving multidrug-resistant organisms (MDROs) occurred after endoscopes were reportedly reprocessed according to guidelines.[10-13] Investigators from the US Centers for Disease Control and Prevention recently concluded that the endoscopes implicated in an outbreak of an MDRO served as an efficient means of transmission, with colonization identified in 39% of exposed patients who were tested in an institution where reprocessing guidelines were followed.[10]

Rapid indicators may be useful in verifying the removal of viable organisms and organic residue from endoscopes during reprocessing.[14-17] Benchmarks for adenosine triphosphate (ATP), protein, carbohydrate, and hemoglobin levels have been established to ensure proper cleaning has been completed prior to endoscope disinfection,[4,18] but these have not been incorporated into current guidelines. A recent validation study determined that thorough manual cleaning decreased bioburden and residual organic debris levels to below the established limits (ie, ATP <200 relative light units [RLU], protein <6.4 μg/cm^2, hemoglobin <2.2 μg/cm^2, and microbial bioburden <4 log10 colony forming units [CFU]/cm^2).[18] Using an ATP indicator, one institution found manual cleaning initially failed to meet established benchmarks 37% of the time. After the establishment of a quality improvement program involving staff training and a revised cleaning algorithm, failure rates after manual cleaning (based on ATP thresholds) were reduced to 5% over 6 months.[19] Because ATP is present in viable organisms and nonviable organic debris, researchers have evaluated the association between ATP levels and microbial cultures.[15,17,20] Both decrease significantly after cleaning.[17,18] A linear association has been described[20]; however, this association is less clear in the context of low microbial burden.[18] As such, measurement of multiple indices of contamination (eg, ATP, protein, hemoglobin, cultures) may be needed to fully characterize residual bioburden on reprocessed endoscopes. The lack of national standards regarding cleaning verification does not provide technicians with any benchmarks to verify cleaning effectiveness.

We sought to determine whether colonoscope and gastroscope contamination caused by clinical use persists despite reprocessing in accordance with current guidelines by performing microbial cultures and rapid indicator tests for ATP, protein, hemoglobin, and carbohydrate residue.

METHODS

Setting

This study was conducted at Mayo Clinic, Rochester, Minnesota, where 30,000 endoscopic procedures are performed annually. Data were collected in an endoscopy unit that reprocesses approximately 100 endoscopes each business day. A waiver was granted by the Mayo Clinic Institutional Review Board because this study did not involve human subjects or protected health information.

Colonoscopy and gastroscopy were performed using Olympus colonoscopes and esophagogastroduodenoscopes (EGDs) (Olympus America, Center Valley, PA), which do not have elevator channels. During this study, reprocessing consisted of several steps. These included bedside cleaning in the procedure room by a technician who flushed enzymatic solution through suction/biopsy (SB) and auxiliary water (AUX) channels and used disposable wipes to clean exterior components. This was followed by leak testing and manual cleaning in dedicated reprocessing rooms. Manual cleaning involved wiping external surfaces, brushing channels and components, and using an irrigation system (Scope Buddy Endoscope Flushing Aid; Medivators, Minneapolis, MN) to flush detergent (Endozime; Ruhof, Mineola, NY) and water through channels. An automated endoscope reprocessor (Medivators SSD-102LT Single Basin AER; Medivators, Minneapolis, MN) was used for HLD (MetriCide OPA Plus; Metrex, Orange, CA). The disinfectant's temperature and minimum effective concentration were verified before cycle initiation. Disinfected endoscopes were stored vertically after drying with isopropyl alcohol and forced air.

Endoscope testing was performed in a dedicated room adjacent to the procedure room, which allowed for rapid sampling and testing. Barrier separation between procedural, reprocessing, data collection, and testing activities minimized potential for environmental cross-contamination. Extensive measures to ensure aseptic environmental conditions during data collection included use of disinfectant wipes on surfaces, use of disposable absorbent pads, and restricting room access. Researchers wore gloves, impervious gowns, face masks with splash protection, hair nets, and shoe covers. Gloves were changed between sampling, and gowns were changed between endoscope encounters.

Sampling

Each instance where samples were obtained from an endoscope was considered an encounter. Samples were collected during a minimum of 4 encounters with each clinically used study endoscope. An endoscope was included when researchers and a technician were available and a GI procedure was completed. Endoscope encounters occurred sequentially after each reprocessing step (ie, bedside cleaning, manual cleaning, HLD) and after overnight storage to assess contamination levels throughout reprocessing. After post-HLD sampling, another cycle of HLD was performed before storage. Components sampled at each encounter included control handles, suction and air and water valves, biopsy ports and caps, distal ends, SB channels, and AUX channels and ports. Tests were conducted to detect protein, carbohydrate, hemoglobin, ATP, and viable microbes.

Visual inspection was performed on all external endoscope components and channel effluent and sampling instruments. External surfaces were individually sampled with sterile swabs. Interior channels were assessed by testing effluent samples obtained via the flush-brush-flush method with 20 mL of sterile water and a 6-mm brush.[18,21] The effluent was divided into 3 sterile collection tubes for microbiologic culturing and rapid indicator testing.

Rapid indicator tests and cultures

Multiple rapid indicators (ie, ATP, protein, hemoglobin, carbohydrate) were used.[18,21] ATP has been validated for assessing endoscope contamination.[17,18] Residual protein is an indicator of inadequate cleaning and can interfere with HLD efficacy.[5] Blood, frequently found within endoscopes after patient use,[16] and sodium ions in blood can inhibit the microbiocidal activity of HLD.[4] Carbohydrate is an energy source for microbes and allows adherence to surfaces.[22]

ATP levels were tested using Clean-Trace Surface ATP and Clean-Trace Water ATP tests (3M, Saint Paul, MN).[23,24] A luminometer quantified ATP expressed in RLU.[20] In accordance with a validated benchmark for clean SB endoscope channels,[18] a cutoff of 200 RLU was applied to evaluate channels and external surfaces. Protein was assessed on control handles and ports (biopsy and air and water) using Clean-Trace Surface Protein-High Sensitivity swabs (3M, Saint

Table 1
Visual inspection of test materials by endoscope component and sampling time

Visual assessment	Bedside cleaning	Manual cleaning	HLD	Storage	Controls	
					New	Automated*
Any component	92 (12/13)	31 (4/13)	0 (0/11)	27 (3/11)	0 (0/1)	0 (0/1)
Individualcomponents						
SB channel	23 (3/13)	0 (0/13)	0 (0/11)	9 (1/11)	0 (0/1)	0 (0/1)
AUX channel	0 (0/7)	0 (0/7)	0 (0/5)	0 (0/6)	0 (0/1)	NA
Suction and air-water ports	0 (0/5)	0 (0/5)	0 (0/3)	0 (0/3)	MISS	MISS
Control handle	54 (7/13)	15 (2/13)	0 (0/11)	9 (1/11)	0 (0/1)	0 (0/1)
Distal end	0 (0/13)	0 (0/13)	0 (0/11)	0 (0/11)	0 (0/1)	0 (0/1)
Biopsy port	39 (5/13)	15 (2/13)	0 (0/11)	18 (2/11)	0 (0/1)	0 (0/1)
Biopsy cap	62 (8/13)	0 (0/13)	0 (0/10)	NA	NA	NA
Suction button	15 (2/13)	8 (1/13)	0 (0/10)	NA	NA	NA
Air-water button	8 (1/13)	0 (0/12)	0 (0/9)	NA	NA	NA

NOTE. Values are percent encounters with contamination (number positive/number total).
AUX, auxiliary water; *HLD*, high-level disinfection; *MISS*, component not sampled; *NA*, not applicable; *SB*, suction/biopsy.
*Reprocessing activities were not directly observed by researchers.

Paul, MN).[25] Protein tests had a lower limit of detection of 10 μg after incubation at 37°C for 15 minutes.[25] Residual carbohydrate, protein, and hemoglobin inside channels were assessed using ChannelCheck dipsticks (Healthmark Industries, Fraser, MI) with effluent. The lower limits of detection for carbohydrate, protein, and hemoglobin were 210, 120, and 0.25 μg/mL, respectively.[26] Positive results from protein swabs or dipsticks indicated contamination because the lower limits of detection were above benchmarks validated for manual cleaning.[16] Per protocol, endoscopes with any positive rapid indicators after manual cleaning were sent back for an additional cycle of reprocessing and repeat testing. If any tests were positive, the endoscope was to be quarantined.

Endoscope channels (SB and AUX) were assessed for aerobic bacteria using Petrifilm Aerobic Count Plates (3M, Saint Paul, MN), a ready-made culturing system designed to expedite colony quantification.[27] For each channel, effluent was used to plate 4 samples for culturing (2 undiluted and 2 diluted 1:10). All plates were incubated at 37°C for 48 hours prior to analysis. After incubation, a Petrifilm Plate Reader (3M, Saint Paul, MN) quantified aerobic bacterial growth by counting CFU on each plate. All positive growth samples identified by the plate reader were sent to a reference laboratory for confirmation and species identification using VITEK 2 Compact (bioMérieux, Hazelwood, MO).

Reprocessing protocol adherence

A reprocessing protocol and checklist were developed a priori in consultation with institution supervisors and based on recommended practices to ensure guideline adherence and standardization of practice.[2,3,28] All reprocessing activities were directly observed by 2 investigators, who used the checklist to ensure protocol adherence.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 21 (IBM, Armonk, NY) and Microsoft Excel 2013 (Microsoft, Redmond, WA). Because the ATP results were positively skewed, medians were calculated instead of means. Fisher exact test was used to compare growth between channels. Mantel-Haenszel summary χ^2 tests were used to calculate the relative risk of persistent endoscope contamination. McNemar test was used to test the association between control handle ATP and protein results after HLD and storage.

RESULTS

Sample size

Researchers collected data during 60 endoscope encounters which occurred November 4-8, 2013. Clinically used colonoscopes (n = 7) and EGDs (n = 6) were sampled. Endoscopes were assessed after bedside cleaning (13 encounters), manual cleaning (13 encounters), HLD (11 encounters), and overnight storage (11 encounters). Ten additional postrecleaning encounters occurred. Samples were also obtained from 2 control endoscopes. A new colonoscope that had never been used (new control) was tested immediately after removal from its packaging to ensure rapid indicators were not producing false positives. Tests were also performed on a control EGD that had undergone cleaning and HLD in a fully automated reprocessor (automated control) using Rapicide PA 30°C High-Level Disinfectant in the Advantage Plus system (Medivators, Minneapolis, MN). Tests were confirmed not to produce false positives because control components had ATP levels below the benchmark and had no detectable protein, hemoglobin, carbohydrates, or viable microbes. The only exceptions were protein and ATP levels exceeding benchmarks on external components handled by technicians (ie, protein on both control handles and ATP on the distal end of the automated control).

Tests included a total of 332 surface ATP tests conducted on distal ends (n = 60), control handles (n = 60), biopsy ports (n = 60), biopsy caps (n = 42), suction buttons (n = 42), air and water buttons (n = 40), and AUX ports (n = 28) (Table 1). Surface protein (75 tests) was assessed on control handles (57 tests) and suction and air and water (18 tests) ports. Researchers tested effluent samples from SB (n = 60) and AUX (n = 28) channels for carbohydrate (n = 88), protein (n = 88), hemoglobin (n = 85), and ATP (n = 88). Aerobic cultures were also performed with effluent from SB (240 samples) and AUX (112 samples) channels.

Interim analysis

Interim analysis on day 2 of data collection determined that contamination levels on multiple endoscopes exceeded post-cleaning protocol-established benchmarks. Unit supervisors, physicians, and researchers amended the protocol to permit additional rounds of cleaning to reduce contamination levels, rather than quarantining endoscopes. The new protocol required retesting after each subsequent attempt to remove contamination, resulting in additional endoscope encounters after a second manual cleaning; a third manual cleaning; HLD after soaking in enzymatic detergent;

Table 2
Rapid indicator and microbial growth findings by endoscope component and sampling time

Sampled component by test performed	Bedside cleaning	Manual cleaning 1	Manual cleaning 2	Manual cleaning 3	Automated*	HLD	Stored	HLD 2†	Controls: New	Controls: Automated‡
Surface ATP§										
Control handle	85 (11/13)	0 (0/13)	0 (0/6)	0 (0/1)	0 (0/1)	0 (0/11)	9 (1/11)	0 (0/2)	0 (0/1)	0 (0/1)
Distal end	100 (13/13)	15 (2/13)	0 (0/6)	0 (0/1)	0 (0/1)	0 (0/11)	18 (2/11)	50 (1/2)	0 (0/1)	100 (1/1)
Suction button	100 (13/13)	23 (3/13)	0 (0/6)	NA	NA	0 (0/10)	NA	NA	NA	NA
Air-water button	77 (10/13)	8 (1/13)	0 (0/6)	NA	NA	0 (0/8)	NA	NA	NA	NA
Biopsy cap	100 (13/13)	46 (6/13)	17 (1/6)	NA	NA	20 (2/10)	NA	NA	NA	NA
Biopsy port	100 (13/13)	46 (6/13)	50 (3/6)	100 (1/1)	0 (0/1)	18 (2/11)	27 (3/11)	50 (1/2)	0 (0/1)	0 (0/1)
AUX port	43 (3/7)	0 (0/7)	0 (0/1)	NA	NA	0 (0/6)	0 (0/6)	NA	0 (0/1)	NA
Water ATP§										
SB channel	100 (13/13)	23 (3/13)	33 (2/6)	0 (0/1)	100 (1/1)	9 (1/11)	9 (1/11)	0 (0/2)	0 (0/1)	0 (0/1)
AUX channel	0 (0/7)	0 (0/7)	0 (0/1)	NA	NA	0 (0/6)	0 (0/6)	NA	0 (0/1)	NA
Surface protein										
Suction and air-water ports	100 (5/5)	20 (1/5)	50 (1/2)	NA	NA	33 (1/3)	0 (0/3)	NA	NA	NA
Control handle	92 (12/13)	75 (9/12)	83 (5/6)	100 (1/1)	100 (1/1)	55 (6/11)	78 (7/9)	0 (0/2)	100 (1/1)	100 (1/1)
Channel dipstick										
SB channel										
Protein	0 (0/13)	0 (0/13)	0 (0/6)	0 (0/1)	0 (0/1)	0 (0/11)	0 (0/11)	0 (0/2)	0 (0/1)	0 (0/1)
Carbohydrate	0 (0/13)	0 (0/13)	0 (0/6)	0 (0/1)	0 (0/1)	0 (0/11)	0 (0/11)	0 (0/2)	0 (0/1)	0 (0/1)
Hemoglobin	38 (5/13)	0 (0/13)	0 (0/5)	0 (0/1)	0 (0/1)	0 (0/11)	0 (0/10)	0 (0/2)	0 (0/1)	0 (0/1)
AUX channel										
Protein	0 (0/7)	0 (0/7)	0 (0/1)	NA	NA	0 (0/6)	0 (0/6)	NA	0 (0/1)	NA
Carbohydrate	0 (0/7)	0 (0/7)	0 (0/1)	NA	NA	0 (0/6)	0 (0/6)	NA	0 (0/1)	NA
Hemoglobin	0 (0/7)	0 (0/7)	0 (0/1)	NA	NA	0 (0/6)	0 (0/5)	NA	0 (0/1)	NA
Aerobic plate growth										
SB channel	79 (41/52)	13 (7/52)	8 (2/24)	0 (0/4)	0 (0/4)	16 (7/44)	2 (1/44)	0 (0/8)	0 (0/4)	0 (0/4)
AUX channel	11 (3/28)	4 (1/28)	0 (0/4)	NA	NA	4 (1/24)	0 (0/24)	NA	0 (0/4)	NA

NOTE. Values are percent encounters with contamination (number positive/number total).
ATP, adenosine triphosphate; *AUX*, auxiliary water; *HLD*, high-level disinfection; *NA*, not applicable (component was not provided or did not exist on the endoscope); *RLU*, relative light units; *SB*, suction biopsy.
*Endoscope was placed in a fully automated reprocessor that performed both cleaning and disinfection.
†Endoscopes were soaked in enzymatic detergent or quarantined in a bucket overnight before receiving another round of HLD.
‡Reprocessing activities were not directly observed by researchers.
§ATP values >200 RLU were considered positive for contamination.

repeated HLD after overnight, quarantined storage; and automated cleaning and HLD in a different hospital department. Six EGDs and 1 colonoscope underwent at least 1 of these additional cleaning or HLD steps, and 1 EGD underwent 4 rounds of vigorous cleaning before quarantine as a result of persistent contamination. In these cases, test results were disclosed to the technician, who recleaned the entire endoscope with special attention paid to components flagged by rapid indicators.

Test results

All reprocessing steps were performed in accordance with guidelines. Visible residue was never apparent to technicians after manual cleaning, and researchers verified that the endoscopes appeared to be clean. However, researchers occasionally observed residue when inspecting effluent and while taking samples with white swabs (Table 1).

After bedside cleaning, rapid indicators detected ATP, protein, or hemoglobin on 13 of the 13 (100%) endoscopes (Table 2). AUX channels and ports had low levels of ATP through all reprocessing steps relative to SB channels and ports. Hemoglobin was detected in 38% of SB channels but not in AUX channels or after any steps other than bedside cleaning. Carbohydrates and protein were never detected by effluent dipsticks after any reprocessing steps. Aerobic cultures showed 92% of bedside-cleaned endoscopes harbored viable microbes. These were predominantly GI flora (eg, *Escherichia coli*, *Enterococcus*, viridans streptococci). Skin flora (eg, *Staphylococcus epidermidis*) were also identified (Table 3). SB channel samples yielded more plates with growth than AUX channel samples ($P < .001$). Of the 52 plates prepared from SB effluent, 25% had colony counts >100 CFU, whereas AUX channel colony counts never exceeded 10 CFU.

After manual cleaning, 12 of the 13 (92%) endoscopes had protein or ATP levels exceeding the benchmarks. Surface protein was detected on 9 of 12 (75%) endoscopes, and ATP levels were above the benchmarks on 9 of 13 (69%) endoscopes or associated buttons or caps. ATP results ranged from 22-1,658 RLU (median, 103) for SB channels, and biopsy ports and caps had the highest ATP results (Table 4). Six endoscopes were sent back for additional cleaning because of high ATP levels. Viable microbes were recovered from 3 of these endoscopes after recleaning and disinfection. Although growth was not observed on most plates, 6 endoscopes (46%) had at least 1 positive culture plate after 1 round of manual cleaning. Colony counts ranged from 1-20 CFU on SB effluent plates (Table 5).

After HLD, rapid indicators were positive for contamination exceeding manual cleaning benchmarks for 8 of 11 (73%) endoscopes. Surface protein was detected on 55% of control handles. SB channels and biopsy ports and caps had the highest ATP levels. One biopsy port had 1,138 RLU, and the corresponding channel and cap had 520 RLU and 319 RLU, respectively. Viable microbes were found in samples representing 64% of endoscopes. Microbes were found in 1 AUX channel (20 CFU, *S warneri*) and 6 SB channels (*Micrococcus luteus*, *Rhizobium radiobacter*, *S epidermidis*, and *S warneri*). All colonoscopes that received only 1 round of manual cleaning ($n = 5$) harbored viable microbes after HLD. ATP levels after HLD ranged from 38-719 RLU (biopsy port or SB channel) for culture-positive endoscopes.

After overnight storage, 9 of 11 (82%) endoscopes had positive rapid indicators. Protein tests from 7 of 9 (78%) endoscopes were positive. Biopsy ports (median, 91 RLU) and control handles

Table 3
Organisms recovered from endoscope channels by sampling time

Channel sampled	Bedside cleaning	Manual cleaning	HLD	Storage
Suction-biopsy channel	*Enterococcus faecalis*, *Escherichia coli*, *Micrococcus luteus*, *Sphingomonas paucimobilis*,*Staphylococcus epidermidis*, *Sta hominis* ssp *hominis*, *Streptococcus*/*Dermacoccus*, *Str gordonii*, *Str haemolyticus*, *Str mitis/oralis*, *Str mitis/oralis/cristatus*, *Str ovis/parasanguinis/sanguinis*, *Str parasanguinis*, *Str pluranimalium*, *Str pluranimalium/Granulicatella adiacens*, *Str pluranimalium/G adiacens/Kocuria rosea*, *Str salivarius*, *Str vestibularis*	*Bacillus cereus/thuringiensis/mycoides*, *Ent casseliflavus*,*Ent gallinarum*, *M luteus*,*Sph paucimobilis*, *Sta warneri*	*M luteus*, *Rhizobiumradiobacter*, *Sta epidermidis*, *Sta warneri*	*Bacillus circulans*
Auxiliary water channel	*Ent faecalis*, *Esc coli*	NA	*Sta warneri*	None

HLD, high-level disinfection; *NA*, not applicable (growth occurred on 1 plate, but species identification was not possible).

Table 4
ATP contamination by endoscope component and sampling time

					Controls (RLU)	
Sampled component	Bedside cleaning (n = 13)	Manual cleaning (n = 13)	HLD (n = 11)	Storage (n = 11)	New (n = 1)	Automated* (n = 1)
Control handle	1,438 (131-22,102)	23 (11-79)	18 (10-117)	52 (15-352)	66	40
Distal end	2,656 (501-85,267)	38 (11-590)	29 (13-48)	41 (14-1,794)	161	794
Suction button	3,929 (461-16,341)	75 (28-616)	37† (19-93)	NA	NA‡	NA‡
Air-water button	389 (110-44,316)	35 (10-700)	19§ (17-38)	NA	NA‡	NA‡
Biopsy cap	64,032 (385-665,977)	183 (11-23,333)	28† (18-319)	NA	NA‡	NA‡
SB channel	1,324 (235-25,954)	103 (22-1,658)	20 (11-520)	34 (12-241)	6	100
Biopsy port	9,029 (643-106,504)	175 (49-5,614)	91 (26-1,138)	37 (20-888)	29	150
AUX channel	6 (3-15)‖	5 (1-10)‖	5 (2-11)¶	5 (2-59)¶	3	NA#
AUX port	112 (36-1,106)‖	22 (13-47)‖	19 (12-28)¶	21 (12-24)¶	24	NA#

NOTE. Values are RLU (range) or as otherwise indicated.
ATP, adenosine triphosphate; *AUX*, auxiliary water; *HLD*, high-level disinfection; *NA*, not applicable; *RLU*, relative light units; *SB*, suction biopsy.
*Reprocessing activities were not directly observed by researchers.
†n = 10.
‡Buttons and caps were not available for testing.
§n = 8.
‖n = 7.
¶n = 6.
#n = 0.

(median, 52 RLU) had the highest ATP levels. Microbes were recovered from the SB channel of 1 of 11 (9%) endoscopes (1 CFU, *Bacillus circulans*).

The relative risk of an endoscope having 1 of the 3 highest ATP results after HLD was 2.1 times higher (95% confidence interval, 1.04-4.11) if it was also 1 of the 3 with the highest ATP results after bedside cleaning ($P = .01$). All 11 endoscopes tested after HLD had control handle ATP measures <120 RLU, but 6 were positive for protein on the control handle ($P = .031$). Similarly, after storage, 8 of 9 had control handle ATP levels <120 RLU, but 7 of 9 were positive for protein ($P = .031$). Viable microbes were recovered after all steps of reprocessing; however, their diversity and prevalence varied by encounter. Small numbers of colonies (frequently <10 CFU) were identified after HLD and storage. It was rare for the same microbe to persist through multiple stages of reprocessing.

DISCUSSION

Residual contamination was detected on multiple components after reprocessing colonoscopes and EGDs in accordance with guidelines. These types of endoscopes do not have elevator wire channels. Although contamination levels generally decreased with each reprocessing step, viable microbes were recovered from patient-ready endoscopes (ie, 64% post-HLD, 9% poststorage). By definition, HLD is expected to eliminate all living organisms, with the exception of some spores.[1] Because all organisms identified after HLD and storage were of low pathogenicity and in low concentrations, they were unlikely to result in patient harm. However, the survival of any organism highlights flaws in the disinfection process that could lead to contamination with and subsequent transmission of pathogenic organisms.

Previous studies show that cleaning endoscope channels is laborious and time consuming, and technicians often skip steps.[29,30] This is concerning, given that the procedure-related risk of endoscopy may be higher than previously thought.[6] Without objective verification, clinicians can unknowingly use contaminated endoscopes for procedures. In this study, reprocessing technicians performed all steps in accordance with guidelines. Current guidelines rely on visual inspection to verify cleaning.[3,5] Visible contamination was never present, potentially because blood and feces may be difficult to discern from endoscopes' black exteriors, and microscopic organisms cannot be seen by the naked eye. In addition, it is impossible to visually inspect interior channels. However, contaminants were often detected via rapid indicators and microbiologic cultures. Results from this study suggest current standards and practices may not be sufficient for detecting and removing all residual contamination.

Transmission of pathogens via gastroscopes,[31] bronchoscopes,[32,33] cystoscopes,[34] and duodenoscopes[10-13,35] has been documented in peer-reviewed literature. Although only colonoscopes and gastroscopes were examined in this study, there are many similarities in the design and reprocessing of other endoscopic instruments for which the results of this study may be

Table 5
Biopsy port and SB channel contamination by endoscope and sampling time

	Bedside cleaning			Manual cleaning			HLD			Storage		
Endoscope identifier by type	Biopsy port ATP (RLU)	SB channel ATP (RLU)	SB channel growth* (CFU)	Biopsy port ATP (RLU)	SB channel ATP (RLU)	SB channel growth* (CFU)	Biopsy port ATP (RLU)	SB channel ATP (RLU)	SB channel growth* (CFU)	Biopsy port ATP (RLU)	SB channel ATP (RLU)	SB channel growth* (CFU)
Gastroscopes												
G1	643	1,324	370	49	111	7	26	42	0	52	50	0
G2	8,520	558	50	374	113	2	151	15	0	35	12	1
G3	68,049	15,434	20	975	963	0	147	78	0	375	182	0
G4	14,389	2,576	4	514	298	20	NA	NA	NA	NA	NA	NA
G5	92,703	25,954	2	5,614	1,658	1	1,138	520	0	888	241	0
G6	106,504	1,467	70	527	103	0	198	61	10	236	93	0
Percent positive for contamination (no./total)	100 (6/6)	100 (6/6)	100 (6/6)	83 (5/6)	50 (3/6)	67 (4/6)	20 (1/5)	20 (1/5)	20 (1/5)	60 (3/5)	20 (1/5)	20 (1/5)
Colonoscopes												
C1	4,036	411	0	175	33	0	70	17	0†	33	14	0
C2	3,633	756	140†	112	27	0	91	11	1	37	56	0
C3	8,030	460	210	2,943	36	0	719	13	1	MISS‡	MISS‡	MISS‡
C4	8,682	235	40	165	32	0	53	13	1	60	13	0
C5	10,925	281	6	118	22	0	MISS‡	MISS‡	MISS‡	22	12	0
C6	15,391	1,402	1,400	79	34	1	46	20	1	29	17	0
C7	9,029	11,064	150†	122	119	0*	38	23	1	20	34	0
Percent positive for contamination (no./total)	100 (7/7)	100 (7/7)	86 (6/7)	14 (1/7)	0 (0/7)	14 (1/7)	17 (1/6)	0 (0/6)	83 (5/6)	0 (0/6)	0 (0/6)	0 (0/6)

ATP, adenosine triphosphate; *CFU*, colony forming units; *HLD*, high-level disinfection; *MISS*, component not sampled; *NA*, not applicable (endoscope did not undergo these reprocessing steps because it was quarantined); *RLU*, relative light units; *SB*, suction biopsy.
*Results represent the highest CFU value obtained from 4 plates.
†Growth occurred on plate(s) obtained from the corresponding AUX channel.
‡Endoscope was not sampled.

applicable. Duodenoscopes used in endoscopic retrograde cholangiopancreatography pose a particular challenge because they have an intricate distal end that accommodates an elevator guidewire needed to allow access to the pancreatic and bile ducts. These components require meticulous cleaning to remove all residual bioburden prior to HLD. Even in cases where technicians did not deviate from reprocessing guidelines, duodenoscopes have harbored MDROs that were transmitted to patients, resulting in colonization, infection, or death.[10-13] In an outbreak in Illinois, 89 of 156 patients exposed to MDRO-contaminated duodenoscopes in 2013 were tested, and 35 patients contracted the organism (9 infected requiring hospitalization and 26 colonized).[10] In 2012, infection prevention staff at a hospital in Pennsylvania traced a significant increase in the incidence of MDRO infections to a contaminated duodenoscope.[11] In a Washington hospital, 35 patients were colonized or infected with an MDRO between 2012 and 2014, and 11 affected patients died.[12,36] In all of these cases, investigators determined that minimum reprocessing standards were followed. In all 3 institutions, investigators cultured endoscope channels and found the outbreak pathogens in addition to other bacteria, including MDROs.

Although they lack elevator guidewires, colonoscopes and gastroscopes are also intricate devices that have multiple channels, ports, and valves. This study demonstrates that colonoscopes and gastroscopes can harbor viable microbes even when adherence with recommended reprocessing guidelines is verified. Further, actions taken beyond guidelines after identifying persistent contamination (additional rounds of manual cleaning and HLD) were not effective. More research is needed to identify processes that can ensure all flexible endoscopes are free of residual contamination and viable microbes prior to patient use.

Knowledge of risk is important to improving clinical outcomes in endoscopy. However, reprocessing technicians often do not have a clear understanding of these risks. Technicians may believe that each step in reprocessing is important, but they may not always perform each step correctly and completely.[29] As indicated by the transmission rates reported in recent outbreaks,[10,35,37,38] the widely cited infection risk estimates are likely highly underestimated. Highly trained reprocessing technicians who rely on this information to guide their practice may believe that endoscopes pose little risk to patient safety.

In the United States, guidelines do not describe benchmarks for contamination on manually cleaned or patient-ready endoscopes. In this study, we used benchmarks established by Canadian researchers for contamination levels deemed acceptable immediately after manual cleaning.[18] Contamination levels found after cleaning, HLD, and storage frequently exceeded these benchmarks. Alfa and Howie[39] stated that buildup biofilm can develop when contaminated endoscopes undergo repeated cycles of HLD and drying. Biofilm provides a protective environment for microbes to survive HLD. The presence of biofilm is suggested by the elevated levels of residual ATP and persistence of microbes in SB channels after reprocessing. Furthermore, channel caps and buttons were found to be highly contaminated, which could recontaminate ports and serve as a source of persistent contamination when reused. To mitigate this risk, more attention should be paid to manual cleaning of all components, including these additional items, which could then undergo sterilization rather than HLD. Alternatively, single-use accessory devices are commercially available. The US Food and Drug Administration has issued draft guidance on this issue.[40]

Assessing contamination prior to HLD allows technicians to reclean endoscopes containing residual debris so that endoscopes with organic residues are not subjected to HLD. The findings from this study suggest multiple components may need to be evaluated because a single component was not found to be an indicator of overall endoscope decontamination. These results also suggest the utility of testing for both protein and ATP because one was often present without the other. This is important, given that commercially available products often have different cutoff points for assessing dirty versus clean surfaces. Neither protein nor carbohydrates were detected in channel effluent using dipsticks, despite detection of contamination by other tests, suggesting that the lower limits of detection may have been too high. Future studies should involve more rigorous culturing methods to identify anaerobes and other microbes that may not have grown using our methods.

Guideline-issuing bodies should review available evidence on endoscopy-associated outbreaks and residual contamination when developing recommendations for verifying reprocessing effectiveness. Organizations in Canada, Europe, Australia, and New Zealand include recommendations on the use of objective measurements to verify that endoscopes are free of contamination, incorporating routine microbial surveillance.[41-44] Conclusions about the association between ATP levels and viable bacterial colonies have been inconsistent,[14,15,17,18,20,45] and the significance of residual protein has not been clearly described. Additional research is needed to determine evidence-based standards for various types of contamination after full reprocessing.

This study was conducted in a single site, and the findings may not be generalizable. Reprocessing technicians were aware of research activities and the use of a checklist to ensure guideline adherence. This awareness and the requirement to reclean contaminated endoscopes based on rapid indicator findings may have influenced the time and effort devoted to reprocessing. Despite these factors, high levels of contamination were found on endoscopes reprocessed during the study. Adherence to current reprocessing guidelines was not sufficient to ensure contamination was completely removed. Furthermore, our results may represent an underestimation of real-world contamination levels, given the generally low adherence to all recommended steps of endoscope reprocessing that has been observed in reprocessing technicians.[9,29,46] The use of rapid indicators allowed technicians to be immediately informed of results whenever benchmarks were exceeded. Additional efforts were made to remove contamination, an action that was not reflective of usual practice. Sampling methodologies (eg, brushing, swabbing, flushing with sterile water) likely removed additional debris, and all disinfected endoscopes underwent an additional round of HLD after sampling. These factors may also have contributed to an underestimation of contamination levels.

Findings from this study suggest testing methods were valid and reliable, as evidenced by contrasting results from clinically used endoscopes and a new, control endoscope. Consistently low contamination levels were also found in AUX channels, which accommodate sterile water during procedures. Further, a steep reduction in contamination occurred from postbedside cleaning to post-HLD. Viable microbes recovered from SB and AUX channels were gut and skin commensal organisms, rather than environmental contaminants, and colony counts decreased through reprocessing stages.

In this study, GI endoscopes were highly contaminated during clinical use, and residual organic materials including viable organisms, persisted despite reprocessing in accordance with guidelines. These results should inform further reprocessing guideline development. Future studies are needed to devise more effective methods of cleaning and disinfecting endoscopes. In the meantime, methods to ensure effectiveness of reprocessing practices are needed, including the potential use of routine monitoring with rapid indicators and microbiologic cultures.

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