Alcohol Flush Does Not Aid in Endoscope Channel Drying but May Serve as an Adjunctive Microbiocidal Measure: A New Take on an Old Assumption

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HIGHLIGHTS

- Alcohol flush (70-30%) prevented outgrowth of *P. aeruginosa* during storage
- Reducing alcohol concentration from 70 to 50 or 30% decreased time to dry channels
- When alcohol flush is omitted:
    - Forced air drying reduced contamination levels and prevented outgrowth
    - Drying beyond the removal of liquid did not eliminate low level contamination
    - Incomplete drying of contaminated channels was akin to no application of forced air

Alcohol Flush Does Not Aid in Endoscope Channel Drying but May Serve as an Adjunctive Microbiocidal Measure: A New Take on an Old Assumption

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ABSTRACT

Background: Alcohol is perceived to aid flexible endoscope channel drying, however we previously showed alcohol increased the time required to dry some channels with forced air versus water alone. Yet, alcohol may prevent microorganism outgrowth during storage. Drying endoscope channels has been shown to prevent outgrowth, but it is unknown if incomplete drying (<10 µl remaining) provides similar protection.

Methods: Endoscope channel test articles were used to determine the efficacy of 70-30% alcohol flush for prevention of *Pseudomonas aeruginosa* outgrowth and drying efficiency. For non-alcohol flushed channels, the impact of forced air drying on outgrowth of *P. aeruginosa* was determined.

Results: Alcohol flush (70-30%) prevented outgrowth with little to no recovery of *P. aeruginosa* during ambient storage. 70% alcohol increased channel drying time by 1.5 or 3-fold compared to 50% alcohol or water, respectively. Forced air drying of non-alcohol flushed channels greatly reduced the initial contamination level and prevented outgrowth. Incomplete drying of contaminated channels was akin to no application of forced air. Applying forced air for more time than necessary to remove residual liquid did not completely eliminate the low level recovery of *P. aeruginosa.*

Conclusions: Flushing with reduced concentrations of alcohol may provide a strategy to prevent microbial outgrowth while reducing drying time.

Keywords: alcohol flush; alcohol biocidal activity; endoscope drying; endoscope reprocessing; endoscope storage; forced air; contaminated rinse water

BACKGROUND

Centers around the world continue to report endoscopically transmitted outbreaks of waterborne and multidrug resistant organisms despite reported adherence to endoscope reprocessing guidelines.(1) This discrepancy may, in part, be due to lack of standardization of critical reprocessing steps. Notably, reprocessing department procedures, professional society guidelines and manufacturer manuals may not provide consistent, detailed information regarding the alcohol flush and drying steps of flexible endoscope reprocessing.(2,3) Current research and progress in reprocessing technology has spurred updates to *ANSI/AAMI ST91: Flexible and Semi-rigid Endoscope Processing in Health Care Facilities*.(4) The key changes include mandatory application of filtered forced air to endoscope channels for a minimum of ten minutes and performing a risk assessment to determine whether alcohol flush should be performed. However, there is no guidance on selection of parameters for drying. Pressure, route of air application (all channels combined with adapters or an air pistol) and type of residual liquid (alcohol or water) have been shown to impact time to dry, with ten minutes not always being sufficient for completely drying endoscope channels.(5)

Performing an alcohol flush has been perceived to facilitate flexible endoscope channel drying, however in a recent study, we showed that alcohol flush does not help to dry the channels.(5) In fact, channels post alcohol flush, retained alcohol during 5 days of storage in an ambient (no directed air flow) endoscope storage cabinet. Moreover, we found that to remove alcohol, forced air (5 to 30 psi) must be applied to endoscope channels, and it can take longer to dry channels containing alcohol compared to those containing water. The qualities of alcohol that aid in rapid evaporation from open surfaces may work against alcohol trapped within narrow

channels. For example, alcohol's lower surface tension, as compared to water, may cause it to flatten and spread rather than being effectively expelled when forced air is applied to the channel. These findings, along with the fixative, flammability and tissue irritant controversies associated with alcohol use, make a strong case for discarding the alcohol flush step.(5) However, there are reports supporting the efficacy of alcohol flush as an adjunctive microbiocidal measure to reduce risk of microbial outgrowth posed by ineffective disinfection, rinse water contamination, or endoscope contamination during storage.(6–8) In addition, 70% alcohol is the broadly accepted concentration for alcohol flush although isopropyl and ethyl alcohol have reported bactericidal activity against Gram positive and negative vegetative bacteria (bacteria implicated in waterborne and multi drug resistant infections) from 70%-30% (v/v).(9,10) The effectiveness of reduced alcohol concentrations for preventing outgrowth of pathogens in channels has yet to be experimentally assessed, and may present a novel strategy for reducing the risks associated with alcohol as well as its negative impact on endoscope channel drying.

The importance of endoscope drying for preventing post-disinfection microorganism outgrowth during endoscope storage(11) or for ensuring effective sterilization prior to low temperature sterilization processes (such as ethylene oxide and vaporized hydrogen peroxide sterilization) is well established.(12) Nevertheless, endoscope manufacturer's instructions may lack specifics on how to effectively dry flexible endoscope channels.(2) Application of forced air is recommended,(13) however we have previously shown that effective drying for each channel system (some flexible endoscopes contain 3 or more channel systems) is unique and impacted by parameters such as air pressure, route of air application and type of residual liquid.(5) Therefore, removing all the residual liquid in endoscope channels is more complex than previously

recognized. Without specific instructions, even with the application of forced air, channels may remain wet.(11,14,15) While the benefits of thorough drying have been previously examined,(11,16) there is an unmet need for research to understand the impact of water remaining in channels due to ineffective drying and to develop strategies for improving drying efficiency.

Here we determined the efficacy of a 70%, 50% and 30% (v/v) isopropyl alcohol (IPA) flush as a measure for preventing outgrowth of the waterborne pathogen, *P. aeruginosa*, in endoscope channels using air/water channel test articles. Additionally in the absence of alcohol flush, the impact of three forced air applications (<10 µL of water remaining, no water remaining and applying air beyond what is required to remove all water) was assessed for controlling outgrowth of *P. aeruginosa* in air/water channel test articles. Lastly, because we previously found that it can take longer to dry channels containing 70% alcohol compared to water,(5) we tested whether reduced concentrations of alcohol (50% and 30% IPA) would be easier to remove from channels to provide a strategy that combines the microbial outgrowth protection of alcohol flush while reducing the time necessary for drying.

METHODS

Protocol for Determining Microbial Levels in Endoscope Channels Post Alcohol Flush and Forced Air Drying

Air/Water Channel Test Articles

Air/water channel test articles were utilized to provide high through-put analysis of the effects of alcohol flush and drying on the outgrowth of a waterborne pathogen, *P. aeruginosa*, in an endoscope air/water channel. The air/water channel was chosen to perform microbial level testing because our previous data shows that narrow diameter channels retain more residual

liquid(5) and contamination may be more prevalent.(17–19) Test articles were used, rather than directly inoculating endoscope air/water channels, to provide a sterile, single use model that eliminated the potential for introducing contamination into actual endoscopes. The test articles were prepared with materials and dimensions that simulated a typical colonoscope air/water channel. The air and water channels were made from 1.2 mm inner diameter (ID) by 1775 mm lengths of clear polytetrafluoroethylene (PTFE) tubing (Cole-Parmer, Vernon Hills, Illinois, United States of America), then joined with a stainless steel "y" connection (from purchased air/water assemblies, Endoscope Development Company, Maryland Height, Missouri) to form a combined air/water channel using a 1.0 mm ID by 185 mm length of clear PTFE tubing (Cole-Parmer, Vernon Hills, Illinois, United States of America). Luer locks were attached to the air and water channels as a mode to deliver forced air or liquid flushes. The test articles were steam sterilized at 132°C for 6 minutes, then the "y" connection area was sealed with "hot-melt" thermoplastic polymer and confirmed airtight by pressurizing under water. The clear tubing allowed for direct visualization of approximately 0.1 µL of residual liquid in the air/water channel test articles to determine the level of channel dryness. Figure 1 shows the steps of preparing the air/water channel test articles.

Pseudomonas aeruginosa Inoculum Preparation

An inoculum of *P. aeruginosa* ATCC 15442 (American Type Culture Collection, Rockville, Maryland, United States of America) was prepared by aseptically streaking a loopful of a defrosted cryostock onto tryptic soy agar plates (TSA, BD Biosciences, Radnor, Pennsylvania, United States of America) and incubating overnight at 37°C. Isolated colonies were transferred to 20 mL of tryptic soy broth (TSB, BD Biosciences, Radnor, Pennsylvania, United States of America), then incubated overnight at 37°C. Optical density at 550 nm was used to estimate the

population of the overnight culture, then a dilution was performed using diluent per EN 16442(20) (composition described in Annex E.1.3.4) to achieve a final concentration of 4 $\log_{10}$ colony forming units (CFU)/mL. The inoculum was serially diluted and enumerated using the drop plate method to confirm the population.(21)

Inoculation and Recovery of Pseudomonas aeruginosa from Air/Water Channels

Air/water channel test articles were inoculated with *P. aeruginosa* to simulate conditions that may occur in a complex channel system of a flexible endoscope if the final rinse step of reprocessing was contaminated with a waterborne pathogen. The standard protocol for "Internal residual contamination of endoscopes after storage" in EN 16442, Annex E(20) was followed for inoculation and recovery of *P. aeruginosa* from endoscope channels. However, the protocol was adapted for testing with the air/water channel test articles. In a biological safety cabinet (enclosed, ventilated workspace), a syringe was attached to the test article's two Luer locks using a plastic "y" and silicon tubing so that both channels were simultaneously attached to the syringe. The distal end of the test article was immersed into the *P. aeruginosa* solution (described above in *Pseudomonas aeruginosa Inoculum Preparation*) and the plunger was drawn up until all sections of the test article were full of inoculum (~4 mL). The syringe was left attached to avoid loss of inoculum while the test article was incubated for 30 minutes under ambient conditions, then the syringe plunger was depressed to remove excess inoculum. To simulate the time between the final rinse step of reprocessing until hanging in a storage cabinet or drying using forced air, the test article was incubated for an additional hour under ambient conditions. Figure 1 lists the steps of inoculating the air/water channels.

For recovery of *P. aeruginosa*, a syringe (adapted using a "y" and tubing to connect to the two proximal ends of the test article) was used to flush the test article. The flushing sequence

consisted of 20 mL of sampling solution (EN 16442, composition described in Annex E.1.3.3) followed by 20 mL of air provided by the syringe (syringe air), then 10 mL of sampling solution and another 20 mL of syringe air. Both flushes were collected in a sterile jar. The samples were enumerated by serial dilution using the drop plate method.(21) To increase the sensitivity of the drop plate method, the jar was rinsed with 20 mL of fresh sampling solution, then the contents were filtered through a 0.45 micron membrane,. The filters were transferred onto TSA (BD Biosciences, Radnor, Pennsylvania, United States of America) and incubated for 2 days at 37°C. Test articles were either recovered immediately (baseline, time = 0) or after treatment according to the methods described in *Impact of Alcohol Flush on the Outgrowth of Pseudomonas aeruginosa in Air/Water Channels* or *Impact of Drying on the Outgrowth of Pseudomonas aeruginosa in Air/Water Channels.*

Impact of Alcohol Flush on the Outgrowth of Pseudomonas aeruginosa in Air/Water Channels

To determine the impact of alcohol flush, inoculated test articles were flushed with 70%, 50% or 30% IPA. In brief, to simulate a typical manufacturer directed manual alcohol flush, a syringe (adapted using a “y” and tubing to connect to the two proximal ends of the test article) was used to flush the inoculated test article with 30 mL of the appropriate concentration of alcohol three times, followed by 30 mL of syringe air three times for a total of 90 mLalcohol and 90 mL air, collecting the flushed liquid in a sterile jar. Flushing with sterile diluent (90 mL total diluent and syringe air) served as a control to differentiate between the mechanical act of flushing and the microbiocidal effects of alcohol. Residual alcohol or diluent remained in the test article after flushing, therefore, one set of test articles, flushed with 70% IPA, were dried with forced air to remove all the residual alcohol to serve as a control for alcohol flush without the lingering effects of alcohol remaining in the test article during storage. The recovery procedure (*Inoculation and*

Recovery of Pseudomonas aeruginosa from Air/Water Channels) was performed on the flushed test articles either immediately (after treatment baseline for the 70% IPA treatment) or after hanging in an ambient endoscope storage cabinet (Stanley InnerSpace Scope Cabinet, Solaire Medical, Grand Rapids, MI) for 48 hours (all flush treatments). A minimum of three replicates were performed for each treatment group.

Impact of Forced Air on the Outgrowth of Pseudomonas aeruginosa in Air/Water Channels

The impact of forced air on the outgrowth of *P. aeruginosa* was assessed in inoculated test articles (*Inoculation and Recovery of Pseudomonas aeruginosa from Air/Water Channels*). Forced air application was performed inside a biological safety cabinet to prevent dissemination of aerosolized *P. aeruginosa*. As a secondary safety precaution, the distal end of the test article was placed inside a biohazard bag while forced air was applied. Application of 5 psi regulated forced air was chosen for this testing because it yielded the following three levels of residual liquid in the air/water channels, depending on the length of time air was applied: 1) <10 µL of liquid remained, 2) all visible liquid was removed or 3) all visible liquid was removed and air application was continued (~ 3 times longer than required to remove all visible liquid). The volume of residual liquid was approximated by measuring the length of liquid filling the tube (volume=$\pi \cdot (r^2) \cdot (l)$, where l = the length of liquid filling tube and r = ½ inner diameter of the channel). After application of forced air, the recovery procedure (*Inoculation and Recovery of Pseudomonas aeruginosa from Air/Water Channels)* was performed either immediately or after hanging the test articles in an endoscope storage cabinet for 48 hours. A minimum of three replicates were performed for each treatment group.

Protocol for Drying Efficiency of Reduced Concentrations of Alcohol

Colonoscope Test Articles

We previously reported that for most endoscope channels, forced air drying took longer when channels contained residual alcohol (post alcohol flush) compared to residual water (post final water rinse).(5) Here, we determined if reducing the concentration of alcohol, thereby increasing water content, would improve drying efficiency.

For this study, a colonoscope test article was prepared as previously described by Nerandzic *et al.*(5) by stripping an Olympus CF-Q180AL colonoscope of its outer sheath, fiber optics and articulation system. The stripped test article simulated the geometry of the internal channel structure and complexity of a full colonoscope, making it possible to perform final water rinse and alcohol flush according to manufacturer's instructions, however the absence of the outer components allowed for direct visualization of liquid remaining inside the channels. To enhance visualization of residual liquid, red or blue food-grade dye (McCormick & Company, Inc, Hunt Valley, Maryland) was used at a ratio of 1-part dye to 1000-parts liquid (v/v) for water rinse and alcohol flush (limit of visual detection increased 2-fold, from 0.1 µL without dye to 0.05 µL with dye). Previous data demonstrated that dye did not affect the efficacy of drying test article channels; there was no statistical difference in drying between dyed versus not dyed liquids.(5)

Preparation of Colonoscope Suction/Instrument Channels

Only the suction/instrument channel of the colonoscope test article was observed in this study. It was chosen as the best model for determining drying efficacy for two reasons: first, our previous studies showed that drying alcohol from the colonoscope suction/instrument channel took statistically longer than drying water,(5) and second, it is the largest diameter channel of the colonoscope and therefore the easiest to visualize residual liquid.

The suction/instrument channel was prepared by performing manual final water rinse or alcohol flush according to manufacturer's instructions. For the final water rinse, the colonoscope test

article was placed in a basin with the manufacturer-issued adapters attached appropriately. Then, the directed volume of water (dyed blue) was applied to the suction/instrument channel systems using a syringe attached to the adapters. Next, the directed volume of air was drawn into the syringe and applied to the channel system through the adapters. External surfaces were dried with lint-free cloths and the valve cylinders and ports were dried with cotton swabs. For the alcohol flush either 70%, 50% or 30% (v/v) isopropyl alcohol (IPA) was prepared, then the suction/instrument channel was processed as described above for final water rinse, however the directed volumes of alcohol (dyed red) and air were applied. The suction/instrument channel contained variable amounts of residual liquid after preparing according to the reprocessing instructions. As a result, the residual liquid remaining in the channel reflected the variability encountered in a real-world setting.

Efficiency of Drying Endoscope Channels After 70%, 50%, 30% and 0% Isopropyl Alcohol Flush

The impact of residual 70%, 50%, 30% and 0% IPA (water), on time to dry the suction/instrument channel was assessed at 15 psi by applying filtered forced air through the suction cylinder on the control handle using a rubber tip nozzle. Application of 15psi regulated forced air was chosen because it was the optimal pressure to visualize liquid removal from the suction/instrument channel. Forced air was filtered through a three-stage filter system (Sharpe Dryaire Desiccant System, Sharpe Manufacturing Company, Minneapolis, Minnesota) and pressure was monitored with a digital pressure gauge (3D Instruments, LLC DTG-6000, WIKA Instruments, LP, Lawrenceville, Georgia).

Three replicates for each of the four residual liquids (70% IPA, 50% IPA, 30% IPA and water) were performed in three blocks with the trials randomized within blocks, for a total of 12 trials.

The results were analyzed with Minitab v.19 software (Minitab, LLC, State College, Pennsylvania) using analysis of variance (ANOVA) and Tukey's pairwise comparisons to determine the statistical significance of residual liquid on time to dry the suction/instrument channel.

To visualize the difference in removal of 70% IPA, 50% IPA and water, a video(22) was recorded during application of forced air to instrument channels containing either 70% IPA, 50% IPA or water (all dyed red to enhance visualization). The instrument channel was inoculated with 5 mL of liquid, then filtered forced air at 15 psi was applied through the instrument port on the control handle using a rubber tip nozzle for 20 seconds. For each liquid a 40 second video(22) was recorded, showing the 20 seconds of air application and 20 seconds for observation of residual liquid.

RESULTS

Impact of Alcohol Flush on the Outgrowth of Pseudomonas aeruginosa in Air/Water Channels

Figure 2 shows the impact of alcohol flush on the outgrowth of *P. aeruginosa* in air/water channel test articles. Air/water channels contaminated with *P. aeruginosa* contained an average of 7.5 $\log_{10}$ CFU after 48 hours of storage in an ambient endoscope cabinet (B; Figure 2, ~3 $\log_{10}$ CFU increase from initial inoculum, A; Figure 2). For alcohol flushed channels, less than 1 $\log_{10}$ CFU (4 CFU recovered in 1 of 3 test articles) of *P. aeruginosa* remained immediately after alcohol flush (C; Figure 2), and no *P. aeruginosa* was detected after 48 hours of storage (D, 70 – 30% IPA; Figure 2). Alcohol flush prevented the outgrowth of *P. aeruginosa* during storage, even when the alcohol was significantly diluted to a final concentration of 30% v/v. Residual alcohol, from the flushing process, remained in the air/water channels during the 48 hours of storage, except for the air/water channels that were flushed with alcohol and then dried with

forced air before storage (E; Figure 2). These samples contained no residual alcohol for the duration of storage and contained no recoverable *P. aeruginosa* after storage. This data demonstrates that residual alcohol in the channel was not required to prevent outgrowth of *P. pseudomonas*. Flushing with sterile diluent removed some of the residual *P. aeruginosa,* but the flushing action alone did not inhibit outgrowth because the diluent flushed channels rebounded by 4 logs after hanging for 48 hours (D, Diluent; Figure 2) compared to channels flushed with alcohol, which showed no outgrowth (D, 70%-30% IPA; Figure 2), suggesting that the mechanism of action of alcohol was primarily antimicrobial activity and not the mechanical act of flushing the channel.

Impact of Forced Air on the Outgrowth of Pseudomonas aeruginosa in Air/Water Channels

Figure 3 shows the impact of applying forced air on the outgrowth of *P. aeruginosa* in air/water channel test articles. There were several factors that impacted the outgrowth of *P. aeruginosa* during the experiments performed in this manuscript. The difference in levels of outgrowth between experiments (comparing B in Figure 2 to B in Figure 3) reflect real world variation that occurs when waterborne pathogens grow under ambient (no temperature or humidity control) conditions and the contamination level is variable. In this experiment, the air/water channels inoculated with *P. aeruginosa* contained an average of 5.5 $\log_{10}$ CFU (~2 $\log_{10}$ CFU less than the alcohol flush experiment) after 48 hours of storage in an ambient endoscope cabinet (~2 $\log_{10}$ CFU increase from initial inoculum, ~1 $\log_{10}$ CFU less than initial inoculum in the alcohol flush experiment). Applying forced air until <10 µL of liquid remained did not prevent or significantly reduce the outgrowth of *P. aeruginosa* in air/water channels; approximately 5 $\log_{10}$ CFUs were recovered after 48 hours of storage (C; Figure 3). Applying forced air until all visible liquid was removed prevented outgrowth and significantly reduced the level of *P. aeruginosa* recovered

after storage to 0.6 $\log_{10}$ CFU (D; Figure 3, recovery of, on average, 4 CFU). Application of forced air ~ 3x longer than the time required to visually dry the channels did not enhance the reduction of *P. aeruginosa* (E; Figure 3).

Efficiency of Drying Endoscope Channels After 70%, 50%, 30% and 0% Isopropyl Alcohol Flush

Table 1 shows the mean times to dry and standard deviations of the various IPA percentages (70%, 50%, 30%) and water (0% IPA) for the colonoscope suction/instrument channel using 15 psi forced air applied through the control handle suction cylinder. Results demonstrated that for the suction/instrument channel system, as the percentage of IPA decreased, the time to dry the channel decreased. The mean drying time for 70% IPA increased by 3-fold compared to water. The percentage of IPA present had a significant impact on the time to dry the suction/instrument channel ($P < 0.0001$), and water was significantly quicker to dry than 70% and 50% IPA, but no statistical difference was seen for time to dry water and 30% IPA.

The video (see link in reference to stream video)(22) shows that the removal of 50% IPA and water is more efficient than removal of 70% IPA after application of 15 psi filtered forced air for 20 seconds. As air is applied to the channel, IPA tends to spread across the channel wall, then recollects into columns of residual liquid when the air application is stopped. In contrast, water is cleanly projected from the channel without spreading and recollecting. Diluting the IPA to 50% enhances its removal from the channel compared to 70% IPA.

DISCUSSION

Previously we showed that, contrary to accepted belief, alcohol flush does not aid in the drying of endoscope channels, especially in the narrow diameter channels which retained residual liquid after 5 days of storage in an ambient endoscope storage cabinet.(5) In addition,

alcohol remaining in channels was more difficult to remove as compared to residual water when using 5 to 30 psi directed forced air to dry the internal channels.(5) While these findings suggest that alcohol flush may not serve the purpose intended for aiding in drying, here we examined another potential role for alcohol flush as an effective measure for preventing outgrowth of a waterborne pathogen in the scenario of contaminated rinse water, ineffective disinfection or contamination during storage. We found that alcohol flush prevented the outgrowth of *P. aeruginosa* in air/water channels during storage, even when the alcohol concentration was reduced to 30% (v/v) (from 70% (v/v)). Without the application of forced air, residual alcohol remains in channels post alcohol flush, but we showed that the microbiocidal effects of alcohol flush persisted after removing residual alcohol with forced air, signifying that residual alcohol does not have to be present in the channel to protect from outgrowth of *P. aeruginosa*.

In the absence of alcohol flush, removing all residual liquid from the channel with forced air followed by hanging in an ambient cabinet for 48 hours did not completely eliminate recovery of *P. aeruginosa* from air/water channels with, on average, 4 CFU recovered. However, it did significantly reduce the initial contamination level by greater than 3 logs and prevented outgrowth (initial contamination level was ~4 $\log_{10}$CFU). These results are in line with what was reported by Alfa and Sitter(23) who showed that drying a duodenoscope instrument channel reduced the occurrence of post-storage contamination from 50% to 0 without an alcohol flush however, the methodology used had a limit of detection of 10 CFU/mL. Further, it was shown that removing most but not all of the residual liquid (<10 µL remaining) in a channel was akin to no application of forced air because the air/water channels contained approximately the same levels of *P. aeruginosa* contamination as seen when no forced air was used (>5 $\log_{10}$ CFU after 48 hours of storage). This result, initially, seemed unexpected. However, when considering that

P. aeruginosa is a strict aerobe, it is likely that the larger residual water "plugs" present in the syringe flushed samples (Figure 3, treatment B) resulted in a much lower growth rate due to lack of oxygen as compared to the growth rate in the very small droplets remaining in the incompletely dried test article (Figure 3, treatment C). In addition, applying forced air for more time than necessary to remove residual liquid did not completely eliminate the low level recovery of *P. aeruginosa*. These findings emphasize the need for development of direct and sensitive drying verification methods to assure optimal drying.

Effective drying of endoscope channels is important to prevent outgrowth during storage post high level disinfection (HLD) processes.(23) For this reason, we tested whether reduced concentrations of alcohol would be easier to remove from channels to provide a strategy that includes the microbial outgrowth protection of alcohol flush while reducing the time necessary for optimal drying. The results demonstrated that the concentration of IPA had a significant impact on the time to dry a colonoscope suction/instrument channel ($P < 0.0001$), and water was significantly quicker to dry than 70%(v/v) and 50%(v/v) IPA, but no statistical difference was seen for time to dry water and 30% (v/v) IPA. Reducing the percentage of alcohol aided in removal from the channel because forced air causes the higher percentage alcohols to spread across the inner surface of endoscope channels, while water is cleanly propelled from the channel likely due to its higher surface tension.(22) The same qualities of alcohol that aid in rapid evaporation from surfaces work against alcohol trapped within narrow channels because the reduced surface tension causes the alcohol to coat the inner channel surface rather than being expelled as is the water. If forced air is not applied for a long enough time to remove or evaporate the alcohol, it will remain, filling sections of the channel and preventing sufficient air exchange for evaporation during ambient storage. Therefore, reducing the concentration of

alcohol by adding water, thereby increasing the surface tension, is an alternative strategy for improving the efficiency of forced air drying.

The use of treated water for endoscope reprocessing is a clear requirement within standard guidelines,(13) however, cases of contaminated rinse water continue to be reported.(24) In a 2020 study by Zhang *et. al*., a pseudo-outbreak of *P aeruginosa* associated with bronchoscopes exposed to contaminated rinse water was reported, highlighting the need for adjunctive measures to control bacterial loads that may develop from water taps or tubing containing biofilms.(24) This example, along with the data presented in this study makes a case for retaining the alcohol flush step in HLD procedures under conditions where effective drying cannot be verified. However, our data shows that the working concentration of alcohol could be reevaluated for several reasons. First, we showed that an alcohol flush, following the final water rinse step at reduced alcohol concentrations, may be effective for preventing contamination of endoscope channels and outgrowth of organisms during storage. Next, reducing the alcohol's concentration mitigates the risks associated with its use, such as flammability, fixative properties or irritation that may occur if introduced to patients' tissues during endoscopic procedures.(5) Lastly, it takes less time to dry endoscope channels containing reduced concentrations of alcohol, compared to higher concentrations of alcohol, therefore increasing drying efficiency.

There are some limitations to our study. First, in this study we assessed the impact of alcohol flush on a Gram-negative waterborne pathogen, *P. aeruginosa*. The pathogens associated with endoscopically transmitted bacterial infections are predominantly vegetative Gram positive and negative organisms. However, alcohol has no sporicidal activity and less biocidal activity at reduced concentrations against some Gram-positive organisms.(9),(10) Further studies are required to determine if reduced concentrations of alcohol are effective for preventing outgrowth

of other Gram positive and negative pathogens.(25) Another limitation is that *P. aeruginosa* strains cultivated in the laboratory may not reflect real world outgrowth and resistance of native strains. Lastly, this study is limited to prevention of contamination occurring from vegetative organisms present in rinse water. Alcohol flush is expected to be less effective in preventing outgrowth of *P. aeruginosa* if the organism was part of an established biofilm within endoscope channels.

CONCLUSIONS

Reduced concentrations of alcohol may provide a strategy to prevent microbial outgrowth while reducing drying time. More research is needed to understand the impact of this strategy using test articles in the presence of residual bioburden (including established biofilms) and, ultimately, evaluating clinically used endoscopes.

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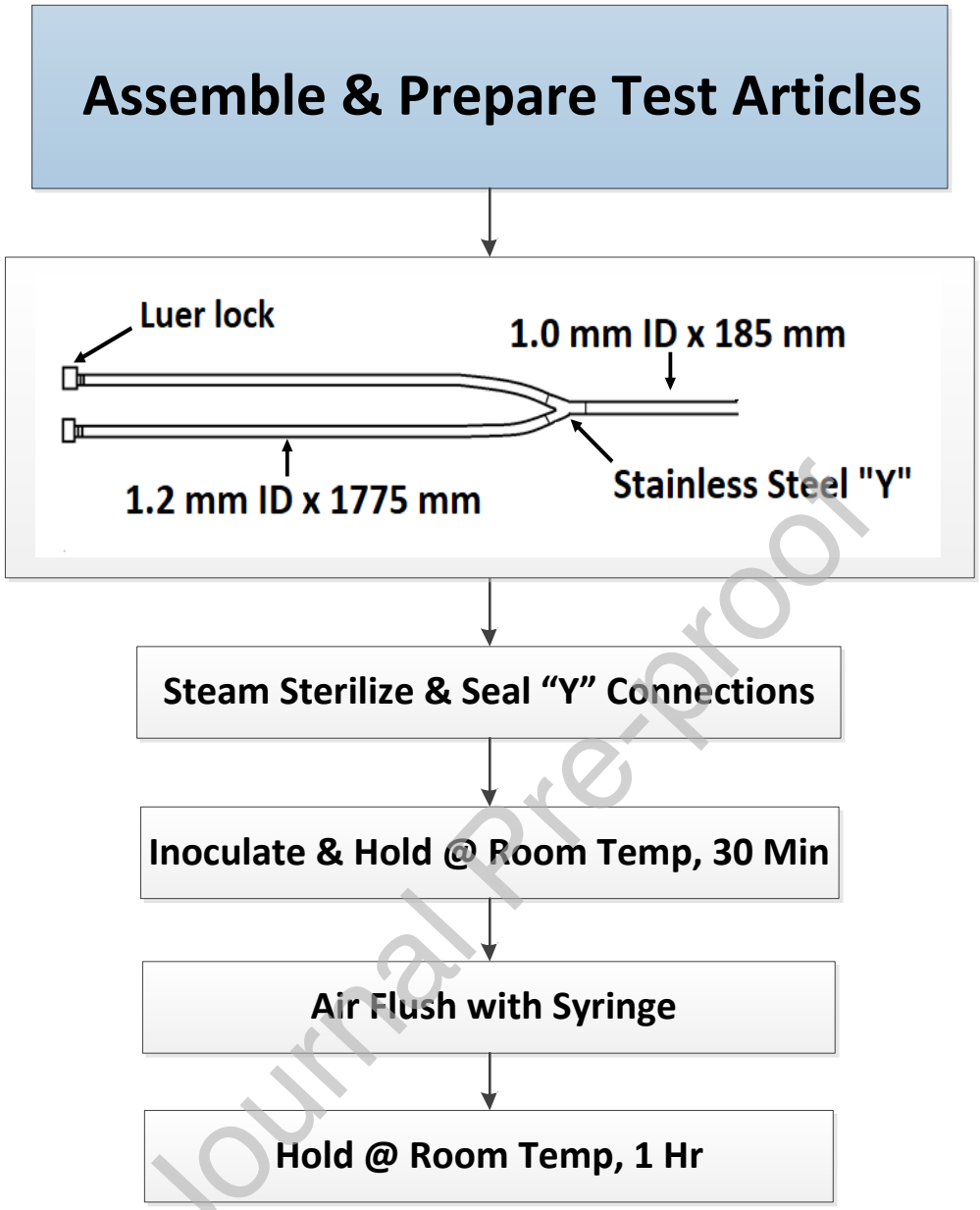

Figure 1. Flow chart of preparation and inoculation of air/water channel test articles.

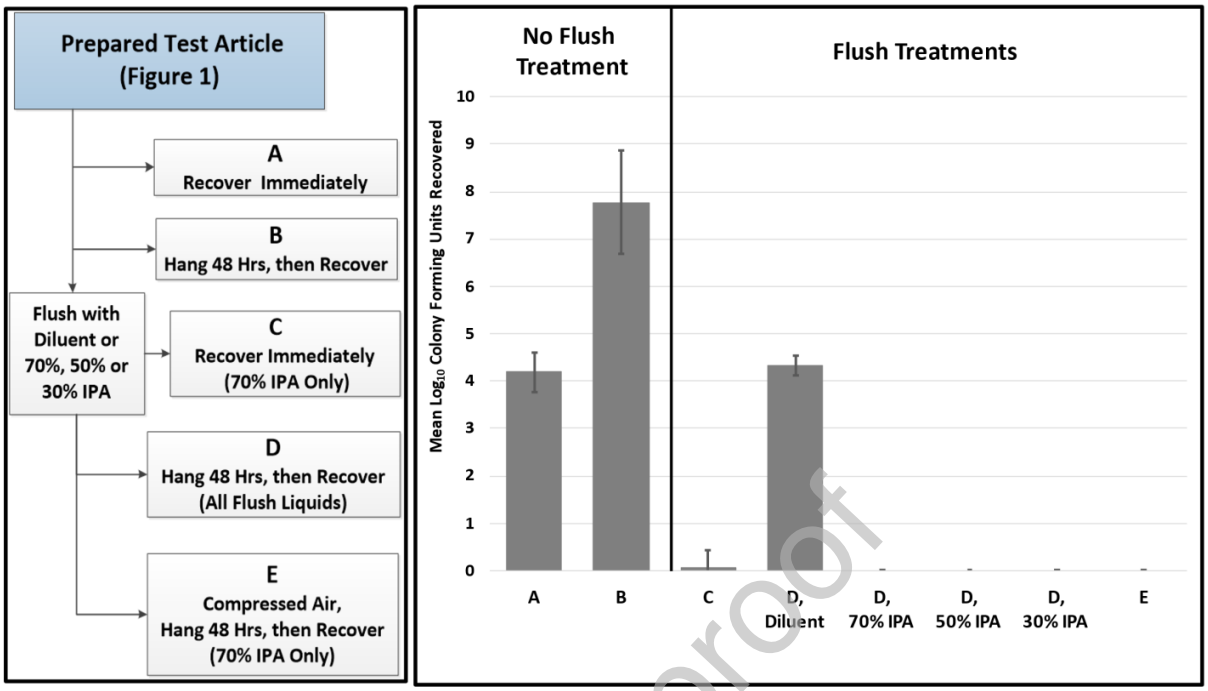

Figure 2. The impact of alcohol flush on the outgrowth of *Pseudomonas aeruginosa* in air/water channel test articles. Air/Water channels were inoculated with approximately 4 $\log_{10}$ colony forming units (CFU) of *P. aeruginosa*. The channels were sampled for *P. aeruginosa* after: inoculation to confirm the initial inoculum, hanging in an ambient endoscope storage for 48 hours, or flushing with the treatments, labeled A-E. The graph shows the mean $\log_{10}$ CFU of *P. aeruginosa* recovered and the error bars represent standard deviation from the mean.

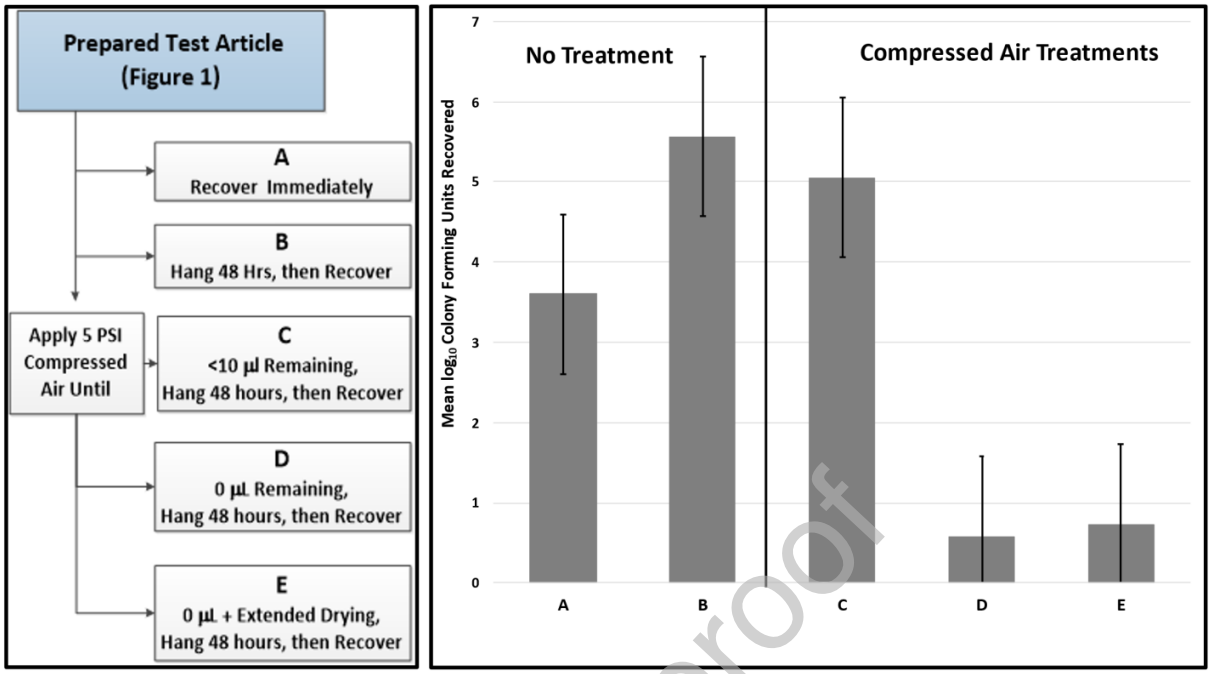

Figure 3. The effects of forced air application until: <10 µL of liquid remained (C), all visible liquid was removed (D) or all visible liquid was removed, and air application was continued ~ 3 times longer than removing all visible liquid (E) on the outgrowth of *Pseudomonas aeruginosa*. Air/Water channels were inoculated with approximately 4 $\log_{10}$ colony forming units (CFU) of *P. aeruginosa*. The channels were sampled for *P. aeruginosa* after: inoculation to confirm the initial inoculum (A), hanging in an ambient endoscope storage for 48 hours (B), or the forced air treatments (C-E) described in the left Panel. The right Panel shows the mean $\log_{10}$ CFU of *P. aeruginosa* recovered and the error bars represent standard deviation from the mean.

Time to Dry the Colonoscope Suction/Instrument Channel System				
Alcohol Percentage	**70**	**50**	**30***	**0***
Mean Time to Dry (min)	27.3	18.9	13.7	8.6
Standard Deviation	1.8	0.9	1.0	3.2

* There was not a statistically significant difference between time to dry 30% alcohol and water from Suction/Instrument Channels

Table 1. The mean time to dry the colonoscope suction/instrument channel containing alcohol at various percentages: 70%, 50%, 30%, and 0% (water). Filtered forced air at 15 psi was applied through the suction cylinder on the control handle using a rubber tip nozzle. Three replicates for each of the four residual liquids (70%, 50%, 30% and water) were performed in three blocks with the trials randomized within blocks, for a total of 12 trials.