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REVIEW

Is peracetic acid suitable for the cleaning step of reprocessing flexible endoscopes?

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Abstract

The bioburden (blood, protein, pathogens and biofilm) on flexible endoscopes after use is often high and its removal is essential to allow effective disinfection, especially in the case of peracetic acid-based disinfectants, which are easily inactivated by organic material. Cleaning processes using conventional cleaners remove a variable but often sufficient amount of the bioburden. Some formulations based on peracetic acid are recommended by manufacturers for the cleaning step. We performed a systematic literature search and reviewed the available evidence to clarify the suitability of peracetic acid-based formulations for cleaning flexible endoscopes. A total of 243 studies were evaluated. No studies have yet demonstrated that peracetic acid-based cleaners are as effective as conventional cleaners. Some peracetic acid-based formulations have demonstrated some biofilm-cleaning effects and no biofilm-fixation potential, while others have a limited cleaning effect and a clear biofilm-fixation potential. All published data demonstrated a limited blood cleaning effect and a substantial blood and nerve tissue fixation potential of peracetic acid. No evidence-based guidelines on reprocessing flexible endoscopes currently recommend using cleaners containing peracetic acid, but some guidelines clearly recommend not using them because of their fixation potential. Evidence from some outbreaks, especially those involving highly multidrug-resistant gram-negative pathogens, indicated that disinfection using peracetic acid may be insufficient if the preceding cleaning step is not performed adequately. Based on this review we conclude that peracetic acid-based formulations should not be used for cleaning flexible endoscopes.

Key words: Peracetic acid; Cleaning; Flexible endoscope; Biofilm; Resistance; Bioburden; Blood; Disinfection; Reprocessing

Core tip: Some formulations based on peracetic acid (PAA) are recommended by manufacturers for cleaning flexible endoscopes. We reviewed 243 studies to analyse the evidence for this recommendation. No study demonstrated that PAA-based cleaners were as effective as conventional cleaners, and some PAA-based formulations had clear biofilm-fixation potential. Dried blood and nerve tissue were substantially fixed by PAA. Some outbreaks, especially of highly multidrug-resistant gram-negative pathogens, indicated that insufficient cleaning could not be compensated for by using PAA in the disinfection step. PAA-based formulations should not be used for cleaning flexible endoscopes.

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INTRODUCTION

Flexible endoscopes come into contact with the mucosa

and are considered as semi-critical equipment, associated with a high risk of infection[1,2]. Infections, including those due to multidrug-resistant gram-negative pathogens, quite frequently occur after gastrointestinal endoscopy[3,4]. The most common types of infections are primary sepsis or bacteraemia[3], pneumonia[3] and gastroenteritis[3], some of which may be fatal. Blood-borne infections such as hepatitis B or hepatitis C have also been described[3]. Most infections are attributed to inadequate cleaning or disinfection of the endoscope before its use on the next patient[3,5,6]. The cleaning process or disinfection step is usually described as inadequate if it deviates obviously from national evidence-based guidelines[7,8].

The processing protocols for flexible endoscopes have changed over the last few decades, with an increase in the popularity of automatic processing[9]. This is associated with advantages such as better standardization, better process validation compared with manual processing[10-17], better overall reprocessing results[18,19] and similar costs[20]. The choice of active disinfection ingredients has increased at the same time. Glutaraldehyde continues to be the main active ingredient in the disinfection step for several decades[21] and is often used for automatic processing at high temperatures such as 56 ℃[22]. It is also used for processing other semi-critical medical devices such as flexible cystoscopes[23], rhinoscopy[24] and bronchoscopes[25]. However, some countries now use peracetic acid-based formulations for the disinfection step[10,14,17,26-30]. Some manufacturers of chemical processing products have recently adapted their processing protocols to recommend the use of peracetic acid-based formulations also for the cleaning step. However, the suitability of peracetic acid for cleaning remains controversial. This study aimed to review the scientific literature on all aspects of the use of peracetic acid-based formulations for cleaning flexible endoscopes, and to provide a clinically relevant summary of the possible implications for patient safety.

STUDY SELECTION

A literature review of the National Library of Medicine was performed on August 19, 2013, using various combinations of the following terms: peracetic acid, cleaning, flexible endoscope, endoscope biofilm, resistance, fixation, infection and outbreak. A total of 471 publications were identified and reviewed for their suitability regarding the topic. A total of 172 studies were considered relevant and evaluated in detail. A further 71 studies not identified by the literature search were also evaluated, *e.g.*, guidelines, reports on side effects, additionally referenced studies or reviews (Figure 1).

STANDARD PROTOCOL FOR PROCESSING FLEXIBLE ENDOSCOPES

Flexible endoscopes are usually processed *via* several steps (Table 1). The cleaning step itself comprises three steps[31]. Pre-cleaning is usually done immediately after use of the endoscope, *e.g.*, with detergent-soaked gauze and rinsing of all channels with the cleaning agents. Pre-cleaning is a standard procedure and may be omitted only under certain conditions[32]. Secondly, brush-cleaning involves cleaning all accessible channels with a brush suited to each channel, and is followed by chemical cleaning, which involves filling all the channels with the cleaning agent for a few minutes, followed by thorough rinsing. The subsequent disinfection step varies in duration, depending on the chemical formulation used and the required spectrum of antimicrobial activity; if virucidal or mycobactericidal activity is required, the duration may be longer. Finally, the endoscope is rinsed once more and dried[33]. Double cleaning is recommended in some countries, such as France, mainly because of the risk of prion diseases[34,35].

The cleaning step itself is considered to be difficult in flexible endoscopes because of the long, narrow lumens and multiple valves[36]. In addition, endoscope channels should be freely accessible, because limited access is associated with significantly poorer cleaning results (approximately 3%)[37]. Manual cleaning is considered less effective than automatic cleaning[38].

IMPORTANCE OF THE CLEANING STEP

There are two major reasons for performing effective cleaning before the disinfection step. First, organic and inorganic materials left on the inner and outer surfaces interfere with the efficacy of the disinfectants[39,40], given that blocked channels may remain undisinfected[41]; only a clean endoscope with clean channels can be disinfected effectively[34]. Second, cleaning of flexible endoscopes aims to reduce the bioburden as much as possible[41]. It is generally acknowledged that the cleaning, rather than the disinfection or sterilization procedure, controls the success of the endoscope[42,43] or angioscopy reprocessing procedure[44] although cleaning alone does not reduce contamination to a safe level[45].

Inadequate cleaning may reduce the efficacy of the disinfection step[46,47] finally leading to contaminated flexible endoscopes after processing, mainly with gram-negative bacteria[48]. Chemical disinfectants work by direct contact between the disinfectant and the microbe, which may be prevented by residual organic material, resulting in incomplete microbial killing[49,50]. Inadequate cleaning was regarded as a main reason in various outbreaks of nosocomial infections associated with bronchoscopy or endoscopic retrograde cholangiopancreatography (ERCP)[51-53]. The importance of optimal cleaning of flexible endoscopes for the overall reprocessing results is acknowledged as a significant issue by physicians and gastroenterology nurses[54].

CLEANING AGENTS

The cleaning agent is usually a detergent without any biocidal ingredient[35]. Some cleaning agents are enzymatic,

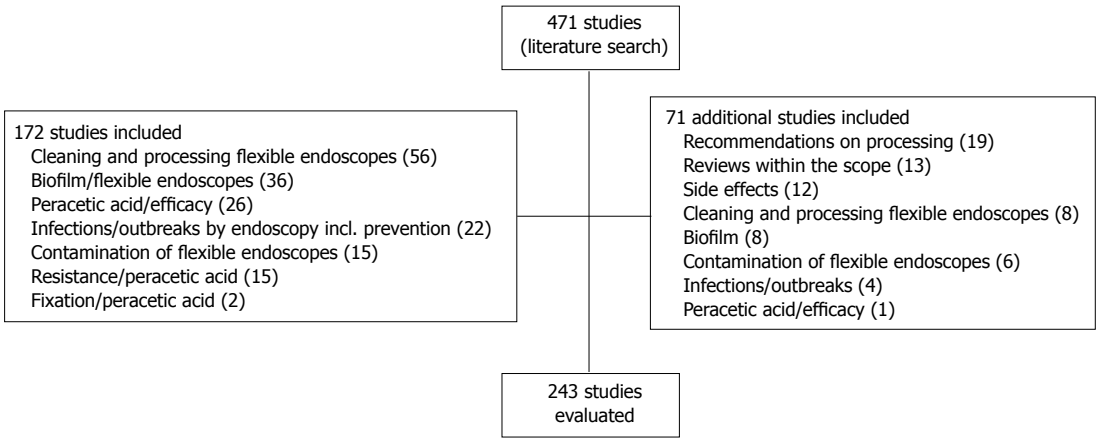

Figure 1 Flow diagram on the study selection process.

Table 1 Typical sequence of steps for manual and automatic reprocessing of flexible endoscopes including the typical duration of the various cleaning steps

Manual processing	Automatic processing
Pre-cleaning the outer surface with a detergent-soaked single-use gauze and rinsing all channels with the cleaning agent, usually for 2 min	
Brush-cleaning all accessible channels with a suitable brush, usually for 3 min	
	Rinsing
Chemical cleaning; filling all channels with the cleaning agent, allowing the cleaning agents to persist inside the channel for approximately 5 min	
Rinsing, usually for 1 min	
Disinfection	
Final rinsing	
Drying	

others are non-enzymatic[55,56]. The cleaning agent should be compatible with the disinfectant agent. The entire process may then achieve a 9 $\log_{10}$ reduction of microorganisms in a tube simulating an endoscope channel[57]. Other processes using different types of cleaning or disinfection agents have revealed lower overall reductions, *e.g.*, a 7 $\log_{10}$ reduction[58]. Lack of use of a detergent in the cleaning step in an automatic processor did not result in any viral blood-borne infections such as hepatitis B or C in 72 patients[59], indicating that the type of cleaning agent is less important in terms of the overall cleaning result for some enveloped blood-borne viruses.

CHEMICAL CHARACTERIZATION OF PERACETIC ACID

Peracetic acid is an oxygen-releasing compound and has been known as a biocidal agent for decades[60-62]. Its current use is mainly for disinfection, *e.g.*, of flexible endoscopes or surfaces[63], sometimes in combination with 1% hydrogen peroxide[64]. In automatic processing of flexible endoscopes, it is used at concentrations of 0.2%[65], 0.35%[66] or even 1%[45], while in manual procedures it may be used at 0.2%[67]. It degrades rapidly to acetic acid and oxygen[68], and its stability is poor compared with glutaraldehyde[69], but may be prolonged by adding stabilizing agents[68]. In common with all oxygen-releasing compounds, it is inactivated by organic materials such as blood[68,70], serum[71,72], albumin[73] or a combination of organic loads[74]. It may be corrosive for a number of materials such as steel or rubber, whereas glass and some plastics are unaffected[68].

FORMULATIONS BASED ON PERACETIC ACID

Various peracetic-acid-based products for processing flexible endoscopes are available in a number of countries; some are powders, and others are liquids used as a one- or two-component system. A number of products available for manual processing are known to the authors and include: Acecide (Saraya Co. Ltd., Osaka, Japan), Gigasept PAA concentrate (Schülke and Mayr, Norderstedt, Germany), neodisher endo DIS active (Chemische Fabrik Dr. Weigert GmbH and Co. KG, Hamburg, Germany), NU Cidex (ASP, Wokingham, United Kingdom), PeraSafe (Antec International Ltd., Sudbury, United Kingdom), Scotalin (KRD, Busan, South Korea), and Sekusept aktiv (Ecolab Inc., St. Paul, MN, United States). Available products for automatic processing include: neodisher Septo PAC (Chemische Fabrik Dr. Weigert GmbH and Co. KG, Hamburg, Germany), Olympus EndoDis (Olympus Europe Holding GmbH, Hamburg, Germany), or Rapicide PA (Medivators Inc. Minneapolis, MN, United States). All these products are described as suitable for the disinfection of flexible endoscopes, but some of them are also recommended by the manufacturer for the cleaning step (Gigasept PAA concentrate, neodisher endo DIS active, and Sekusept aktiv).

PATHOGENS

Pathogens on flexible endoscopes after use

The total contamination of flexible endoscopes with pathogens is usually highest in colonoscopes, followed by gastroscopes and bronchoscopes[75]. The microbial load

after patient examination was found to be between > 10^3 and 10^{10} colony-forming units (CFU) per milliliter[48,76], with highest numbers in the suction channel[77-79]. The contamination consisted mainly of gram-negative bacteria (56%) such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*, followed by gram-positive bacteria (27%) such as *Staphylococcus aureus*, coagulase-negative *Staphylococcus* and *Micrococcus luteus,* and yeasts (17%) such as *Candida albicans* and *Candida tropicalis*[48]. The air and water channels may, however, also be contaminated[80]. If biopsy suction channels are not adequately cleaned, remaining pathogens may contaminate single-use sterile biopsy forceps during passage[81,82].

Infected patients leave their infectious flora on the endoscope. Hepatitis B virus DNA, hepatitis C virus RNA, human immunodeficiency virus DNA and *H. pylori* have been found after use of endoscopes in infected patients[83-86], especially in the biopsy suction channel[87], and even after cleaning[88]. It is estimated that, on average, 4 in every 1000 endoscopies result in transmission of *H. pylori*[89].

Pathogens on flexible endoscopes after cleaning

The cleaning step can reduce the bioburden by 4.7 $\log_{10}$ CFU (gastroscopes) and 6.2 $\log_{10}$ CFU (colonoscopes)[76,90]. Automatic cleaning and manual cleaning resulted in a similar reduction in microbial load (4.32 and 4.24, respectively), when measured with *E. faecalis* and *P. aeruginosa*[33]. *M. chelonae* may be reduced by 4 $\log_{10}$-steps by standardized manual cleaning[91]. Automatic cleaning processes may achieve a $\log_{10}$-reduction of 7.0-8.4, depending on the type of washer disinfectant and cleaning agent[92].

In contaminated test tubes the cleaning step during automatic processing of flexible endoscopes shows variable results, depending on the type of process and the cleaning agent[58]. Some cleaning processes using a detergent were significantly less effective (0.3 $\log_{10}$-steps) than water alone (1.1-2.6 $\log_{10}$-steps), indicating that the entire cleaning process needs to be evaluated critically[55,56]. In contrast, other cleaning processes were significantly more effective (4.1 $\log_{10}$-steps)[56].

HCV is usually completely removed from the biopsy suction channel by the cleaning step alone, as demonstrated in 19 upper gastrointestinal endoscopic procedures in patients with chronic replicative hepatitis C[85]. This finding is supported by *in vitro* data using contaminated high-titre HCV-positive plasma for experimental contamination of flexible endoscopes[93], and by evaluation of flexible endoscopes used in patients with hepatitis C[94]. HIV was a also reduced by at least 99.93% using a detergent cleaning step alone[95].

Overall cleaning effectively reduces or eliminates many pathogens by at least 4 log as recommended[77], but substantial levels of viable bacteria may remain[78]. This suggests that the risk of transmission of nosocomial pathogens cannot be eliminated by cleaning alone[96]. Poor mechanical cleaning may be indicated by a high titre of microorganisms in a surveillance culture[97].

Effect of peracetic acid on pathogens

Antimicrobial activity: Peracetic acid is very reactive and has strong antimicrobial activity. Depending on its concentration and pH value[98], it is effective against bacteria including *H. pylori*, fungi, mycobacteria, viruses including hepatitis B virus, and bacterial spores[35,66,68,99-112], though for specific isolates, such as *Mycobacterium gordonae*, the exposure time may have to be prolonged to 20 min to achieve the required efficacy[67]. However, despite its broad spectrum of antimicrobial activity it is not suitable for sterilizing surgical instruments[113]. In combination with copper, peracetic acid is also considered to be suitable for prion decontamination[114]. The optimal pH value for its antimicrobial activity is between 2.5 and 4[68]. It is also assumed that exposure of gram-positive species such as *Bacillus subtilis* to chlorine dioxide enhances a stable cross-resistance to other oxidizing agents, such as peracetic acid[74], as confirmed by Bridier *et al*[115]. The efficacies of different formulations differ remarkably compared with solutions of the active ingredient alone[116].

Cellular changes to sublethal concentrations: Bacterial resistance to biocides is apparently increasing, although peracetic acid has not been implicated in the selection and persistence of bacterial strains with low-level antibiotic resistance[117]. Exposure of nosocomial pathogens to peracetic acid at a sublethal concentration (*e.g.*, 1 mmol/L) has been reported to induce a cellular response in *S. aureus*. This response includes the induction of many virulence-factor genes upon exposure, suggesting stimulation of pathogenesis in response to peracetic acid[118]. Other effects included significant alterations in the regulation of membrane-transport genes, selective induction of DNA-repair and -replication genes, and differential repression of primary metabolism-related genes between the two growth states[118]. Similar reactions were observed after exposure of *P. aeruginosa* to a sublethal concentration (*e.g.*, 1 mmol/L) of peracetic acid: many genes associated with cellular protective processes were induced, while transcription of genes involved in primary metabolic pathways was repressed, and that of genes encoding membrane proteins and small molecule transporters was altered[119]. In terms of *E. coli* O157:H7, a sublethal concentration of peracetic acid (0.1%) induced a substantial increase in peroxidative tolerance[120]. Finally, a strain of *Salmonella typhimurium* exposed to a sublethal concentration of peracetic acid (*e.g.*, 15 mg/L) showed modified physiological characteristics: the cells remained viable but were unable to be cultured, but retained their virulence, as shown by their adhesive and invasive capacities[121]. A higher concentration of peracetic acid (*e.g.*, 20 mg/L) resulted in bacterial death. This study indicated that a negative culture result from an endoscope does not exclude the presence of pathogens on the endoscope, and transmission may occur if the bacterial cells modify their physiological characteristics, *e.g.*, by exposure to sub-

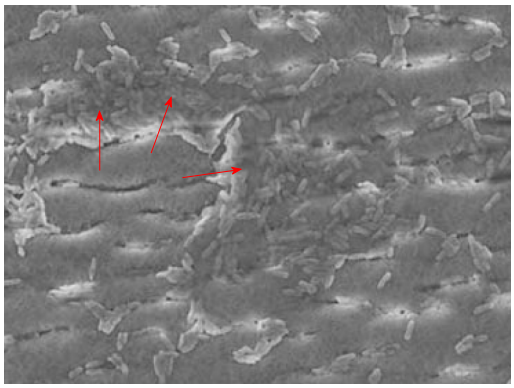

Figure 2 Residual biofilm after exposure to 0.09%-0.15% peracetic acid, as shown by Balsamo *et al*[141]. Reproduced by kind permission of the publisher.

lethal concentrations of peracetic acid.

BIOFILM

General background

Biofilms are communities of cells that are attached to an abiotic or living surface embedded in an extracellular polymeric substance[122,123]. They are preferentially formed in wet environments (*e.g.*, insufficient drying of endoscopes before storage[124,125]), can form under different flow conditions[126,127] and can be potential sources of contamination and infection[128]. Virtually all bacterial species can form biofilm including clinically-relevant ones such as *P. aeruginosa*, *S. aureus*, *E. coli* and *Clostridium difficile*[123,129,130]. Under natural environmental conditions, biofilms are likely to be composed of a mixture of different species[131,132]. In the laboratory, they can be grown on various materials and devices, including polystyrene microtitre plates[133-136], haemolysis glass tubes[137,138], stainless steel coupons[134,139] and also in Teflon tubes[140-143], similar to endoscope channels.

Resistance of biofilm bacteria

One feature of many biofilm bacteria is their resistance to some antibiotics and disinfectants ([144-147] and reviewed in[148,149]). Artificial *P. aeruginosa* biofilms resisted treatment with various biocidal agents including peracetic acid, compared with their planktonic counterparts[150-152]. Biofilms composed of *E. coli*[152,153], *S. aureus*[152,154,155], *Mycobacterium fortuitum*[156] or *Listeria monocytogenes*[157] also resisted treatment with diverse biocides compared with planktonic cells. Bacteria in mature (old) biofilms were more resistant to killing than those in young biofilms[153,158,159]. An older biofilm of *P. aeruginosa* required up to 20-fold higher concentrations of peracetic acid (0.2%) to be eradicated, compared with their planktonic counterparts (0.01%)[151]. Similar results were found with an *E. coli* biofilm and peracetic acid/H_2O_2[153]. The resistance of biofilms can often further increase when the communities are composed of more than one bacterial species[134,136,160-163] which may include resistance against 0.35% peracetic acid, which is a concentration used in many formulations[133]. Especially "build-up" biofilms mimicking repeated endoscope reprocessing cycles exhibited a significantly higher survival rate than 'traditional' biofilms[158]. The mechanisms underlying disinfectant-resistant phenotypes appear to be multifactorial[133,148,151,153,164].

Biofilm on flexible endoscopes

Direct evidence for extensive biofilm contamination was provided in 1 of 13 investigated biopsy suction channels and 5 of 12 air/water channels of reprocessed endoscopes[165]. Some reports showed persistent levels of bacteria in endoscope channels, despite reprocessing according to published guidelines, providing indirect evidence for contamination by biofilms[166-168]. Residual biofilm can be seen in Figure 2. In one case, a colonoscope was contaminated with a total of 195 bacteria despite six rounds of reprocessing[168]. Treatment with a cleaning agent that had previously been shown to remove biofilms from endoscope tubes[142] was capable of eradicating the microbes almost completely, indicating that the presence of biofilm was the main reason for ongoing bacterial contamination[168]. Biofilms were also found in washer disinfectors resulting in contamination of automatically-processed endoscopes, *e.g.*, with *Mycobacterium chelonae*[169,170], *Methylobacterium mesophilicum*[170] or *P. aeruginosa*[171], some giving rise to nosocomial infections[171]. Biofilm formation and fixation should therefore also be avoided in washer disinfectors[172]. If biofilms are not thoroughly removed from endoscope channels by cleaning, subsequent disinfection might fail, enabling microorganisms to persist. Further, efficient interchange of plasmids might occur in biofilms, including those coding for antibiotic resistance such as cefotaxime- or aminoglycoside-resistance[173-176].

Biofilm on flexible endoscopes after cleaning

Shear stress was found to remove some biofilms, though 24% and 47% of the biofilm masses, respectively, remained attached[177]. Brushing a silicone tube 10 times with a sterile brush was found to completely remove a multispecies biofilm that had developed over a period of 50 d[178].

Commercial detergents show variable results on biofilm removal[179]. A non-enzymatic detergent yielded a significantly higher $\log_{10}$-reduction (4.13 to 4.17 $\log_{10}$-reduction) of residual wall *E. coli* biofilm bacteria than the enzymatic detergents (0.74 to 0.88 $\log_{10}$-reduction), whilst contact time (3, 5 or 7 min) had no significant impact[180]. Similar results on different cleaners were reported by Fang *et al*[181] and Vickery *et al*[182]. Quantification of endotoxin levels also revealed better results for a non-enzymatic cleaner in terms of biofilm reduction[183]. A non-enzymatic cleaner continued to remove more biofilm with an increasing number of wash/contamination cycles: by the 20th cycle, 90% of the tubing was biofilm-free[184].

New cleaning formulations based on phosphates, hydrates, minerals and surfactants were developed several

years ago[142]. These formulations effectively removed multispecies biofilms from Teflon tubes, prevented the growth of new biofilms in endoscopes, and established biofilms were completely removed from endoscopes by sequential washing with an enzymatic solution and a bleach-enriched version of the new cleaning formulations[142]. Three repeats of a reprocessing of more than 1 h using sequential application of these cleaning components almost completely removed biofilms from flexible endoscopes that had been used in patients, and were persistently contaminated with bacteria despite six rounds of reprocessing[168]. The practicality of this procedure, however, remains doubtful.

Effect of peracetic acid on biofilm

Treatments with aldehyde, peracetic acid plus detergent, or chlorine failed to disturb or remove biofilm, despite a significant log reduction in biofilm bacteria[178]. Biofilm in a water line in a dental unit with permanent water contact was effectively removed by a peracetic acid flush (0.26%)[185], but this has no correlate in endoscope processing. *P. aeruginosa* biofilms remained in an endoscope prototype in 76.2% of tested tube segments after cleaning followed by manual peracetic acid (0.09%-0.15%) processing and in 23.8% after cleaning followed by automatic peracetic acid processing[141]. The same processes with glutaraldehyde (2%) revealed lower rates of 71.4% after manual processing and 4.8% after automatic processing[141]. Protein in a *P. aeruginosa* biofilm could be removed by peracetic acid by 41%. The removal is much lower from mature biofilms or biofilms subjected to repeated peracetic acid treatments, which may modify biofilm structure[143]. At the same time, the biofilm was partially fixed and accumulated after exposure to two peracetic acid-based formulations[143]. Fixation rates varied between formulations within the same chemical group[143]. Four peracetic acid-based products were reported, two of which fixed artificial biofilms quite strongly, while the other two containing additional quaternary ammonium compounds showed no biofilm fixation[138]. An *E. coli* biofilm exposed to three different peracetic acid-based formulations (one with peracetic acid, one with additional non-ionic surfactant, and one with additional cationic surfactant) was partly removed by two formulations, and not fixed by any of the three formulations[137].

Finally, sublethal concentrations of chlorine dioxide, an active compound used for disinfection of endoscopes, *may accelerate* formation of *B. subtilis or P. aeruginosa biofilms* compared with biofilms grown in the absence of chlorine dioxide[186]. A similar effect can be expected with other oxygen-releasing compounds.

BLOOD

Blood on flexible endoscopes after use

Contamination of flexible endoscopes with blood is to be expected, *e.g.*, after biopsy or in the case of variceal gastrointestinal bleeding. It is also common in other types of endoscopic procedures[187]. After different types of endoscopic procedures, suction channels contain haemoglobin at a concentration of 85 μg/cm^2[78]. Residual blood may contain blood-borne viral pathogens[83,84,87,88] and may impair the efficacy of the subsequent disinfection step[44,68,70,188].

Blood on flexible endoscopes after cleaning

Detergent-based formulations are capable to remove between 88% and 95% of dried blood while peracetic acid-based formulations only removed 8%-59% depending on the type of formulation[183,189]. These results indicate that dried blood is not removed as easily by peracetic acid-based formulations compared with detergent-based formulations.

Effect of peracetic acid on blood

At the same time, however, the rate of fixation of blood exposed to the same peracetic acid-based formulations was between 19% and 78%[189], indicating that the remaining blood is fixed and cannot be easily removed. A similar effect can be seen on clinically used endoscopes containing organic contamination fixed by glutaraldehyde disinfectant solution: 20 cleaning cycles using a buffered peracetic acid procedure removed 30%-50% of the contamination[190]. These data highlight the need to avoid contact between organic contaminant and agents with fixation properties, because subsequent removal may be difficult.

OTHER ORGANIC CONTAMINATION

Organic contamination on flexible endoscopes after use

Suction channels may contain proteins at a concentration of 115 μg/cm^2 after endoscopic procedures[78].

Organic contamination on flexible endoscopes after cleaning

Organic contamination may remain after cleaning. It was reported that 95 out of 504 samples obtained before disinfection and tested for adenosine triphosphate were above the benchmark values (200 relative light units [RLUs])[191], indicating inadequate cleaning[192]. Levels may be as high as 10417 RLUs on the exterior endoscope surface, or 30281 RLUs on the biopsy suction channel rinsates[193].

Haemoglobin and protein may also remain after cleaning. A channel is considered clean if the haemoglobin level is < 2.2 μg/cm^2 and the protein level is < 6.4 μg/cm^2[194]. If all these parameters are fulfilled, the ATP level will be < 200 RLUs[191] which can be considered a validated benchmark from patient endoscopes[195].

Overall, most of the organic contamination is usually removed below benchmark by detergent-based cleaning procedures, although exceptions may occur[196].

Effect of peracetic acid on organic contamination

Peracetic acid used for high-level disinfection of duo-

Table 2 Outbreaks and pseudo-outbreaks reported in connection with biofilm or peracetic acid-based processing of flexible endoscopes

Number/type of infection(s)	Pathogen(s)	Type of endoscopic procedure	Reason for outbreak / pseudo-outbreak	Peracetic acid-based formulations were used for	Ref.
None (pseudo-outbreak)	*Pseudomonas aeruginosa*	Gastroscopy, bronchoscopy	Suboptimal duration of glutaraldehyde application during disinfection; "resistance" to glutaraldehyde may have been enhanced by manual cleaning with peracetic acid-based disinfectant[214]	Cleaning step	[202]
2: infection (not further specified) 3: colonization	OXA-48 *Klebsiella pneumoniae*	Bronchoscopy	A problem with the washer disinfector or the cleaning procedure was assumed as the reason	Cleaning step and disinfection step (Gastmeier P, personal communication)	[203]
4: pneumonia (3 cases); colonization (1 case)	MDR *Pseudomonas aeruginosa*	Gastroscopy	Insufficient initial cleaning, shortening of the immersion time and brushing time, insufficient channel flushing, and inadequate drying prior to storage	Disinfection step	[124]
4: bacteraemia, biliary tract infection, respiratory tract infection 9: colonisation	KPC-2 *Klebsiella pneumoniae*	Duodenoscopy	Contaminated duodenoscope; reason for outbreak: inadequate cleaning	Disinfection step	[204]
8: bloodstream infection 4: biliary tract infection 4: colonization	ESBL *Klebsiella pneumoniae* (CTX-M-15)	ERCP	Insufficient manual cleaning, insufficient drying after processing	Disinfection step	[125]
3: sepsis	*Pseudomonas aeruginosa*	ERCP	Presence of biofilm on undamaged channels	Disinfection step (Kovaleva J; personal communication)	[205]
5: infection (not further specified) 9: colonization	OXA-48 *Klebsiella pneumoniae*	Duodenoscopy	One endoscope had probably a defect resulting in insufficient disinfection	Disinfection step (Gastmeier P, personal communication)	[203]
18: pulmonary infection (4 cases, one of them died); colonization (14 cases)	Imipenem-resistant *Pseudomonas aeruginosa*	Bronchoscopy	Incorrect connectors joining the bronchoscope suction channel to the STERIS SYSTEM 1 processor	"Automatic processing"	[206]
2: bacteremia and biliary tract infection 4: colonization	KPC-2 *Klebsiella pneumoniae*	Gastroscopy	Delayed pre-wash resulting in drying of the gastroscope; short drying period after the peracetic acid treatment resulting in incomplete drying	"Wash"	[207]

ERCP: Endoscopic retrograde cholangiopancreatography.

denoscopes yielded significantly lower levels of protein (4.2 μg/mL *vs* 10.1 μg/mL), carbohydrate (18.5 μg/mL *vs* 111.1 μg/mL) and endotoxin (2.8 EU/mL *vs* 44.5 EU/mL) in the biopsy suction channels compared with processes using glutaraldehyde[197]. Despite the differences between the two active agents used only for the disinfection step, the authors concluded there may be a cumulative build-up of organic material components on the inner lumen of the biopsy suction channels of endoscopic retrograde cholangiopancreatography scopes in use[197]. An outbreak of eight fatal cases of *Serratia odorifera* septicemia was caused by contaminated parenteral nutrition fluid due to inadequate cleaning of the surfaces prior to the use of peracetic acid[198]. Dialyzers cleaned with peracetic acid showed significantly lower clearance of larger dextrans as a result of the presence of residual proteins on or within the membrane[199]. Similar findings were reported with a product containing hydrogen peroxide and peroxyacetic acid, compared with one containing sodium hypochlorite[200].

Special case: effect of peracetic acid on nerve tissue

Exposure of brain homogenate to peracetic acid (1500 ppm for 20 min) is associated with a very high protein fixation rate of 96%, which is much higher than with exposure to glutaraldehyde (19%)[201]. Mice inoculated with variant Creutzfeld-Jacob disease (vCJD)-infective brain homogenate previously exposed to peracetic acid survived on average 291 d, which was significantly shorter than mice inoculated with negative control homogenate (> 450 d). Mice inoculated with vCJD-infective brain homogenate previously exposed to glutaraldehyde (2% for 20 min) survived longer compared with the peracetic acid group (mean: 324 d), demonstrating a clinical correlate of the almost complete fixation of brain homogenate protein by peracetic acid[201].

OUTBREAKS AND PSEUDO-OUTBREAKS

Outbreaks and pseudo-outbreaks connected with peracetic acid-based processing of flexible endoscopes are summarized in Table 2. In some outbreaks peracetic acid was used for the cleaning step[202], the cleaning and disinfection step[203], the disinfection step[124,125,203-205] or generally for processing/washing[206,207]. The reasons for the infections were insufficient (initial) cleanin[124,125,202-204], inadequate drying prior to storage[124,125,207], shortening of the immersion time and brushing time[124], insufficient channel flushing[124], a problem with the washer disinfec-

Table 3 Adverse effects after processing with peracetic acid after endoscopy

Number of cases	Type of reaction	Possible explanation	Ref.
10	Colitis	Unclear, reprocessing with PAA, but afterwards channels were flushed with hydrogen peroxide	[210]
1	Colitis	PAA residues in the biopsy suction channel	[215]
2	Colitis	Defect of automatic rinsing of a channel	[216]
1	Colitis	Channel not flushed	[217]
1	Colitis	Inadequate rinsing of a channel	[212]
No number provided	Pseudolipomatosis	Air channels not rinsed	[218]
4	Colitis	Programming error in the automatic disinfection device, related to the air/water channels	[219]
12	Colonic mucosal pseudolipomatosis	Rinsing was not done as recommended	[220]

Table 4 Overview of evidence-based guidelines for processing flexible endoscopes, focusing on the use of peracetic acid during the cleaning step

Institution	Guidelines	Year	Use of peracetic acid for cleaning
AORN	Recommended practices for cleaning and processing endoscopes and endoscope accessories[221,222]	2012	No recommendation
APIC	APIC guidelines for infection prevention and control in flexible endoscopy. Association for Professionals in Infection Control[223]	2000	No recommendation
APSIC	The ASEAN Guidelines for disinfection and sterilization of instruments in health care facilities[224]	2012	No recommendation
ASGE	Multisociety guidelines on reprocessing flexible gastrointestinal endoscopes: 2011[225,226]	2011	No recommendation
BC Ministry of Health	Best Practice Guidelines For Cleaning, Disinfection and Sterilization of Critical and Semi-critical Medical Devices[227]	2011	No recommendation
BSG	BSG Guidelines for Decontamination of Equipment for Gastrointestinal Endoscopy[228]	2008	No recommendation
CDC	Guidelines for Disinfection and Sterilization in Healthcare Facilities, 2008[229]	2008	No recommendation
ESGE/ESGENA	ESGE/ESGENA Technical Note on Cleaning and Disinfection[230][1]	2003	**Recommended**
ESGE/ESGENA	ESGE-ESGENA guideline: Cleaning and disinfection in gastrointestinal endoscopy, update 2008[231]	2008	No recommendation
HPS	Endoscope Reprocessing: Guidance on the Requirements for Decontamination Equipment, Facilities and Management[232]	2007	No recommendation
JGETS	Guidelines for cleaning and disinfecting endoscopes - Second edition[233]	2004	No recommendation
Public Health Agency of Canada	Infection Prevention and Control Guideline for Flexible Gastrointestinal Endoscopy and Flexible Bronchoscopy[234]	2010	No recommendation
RKI	Hygiene requirements for reprocessing of medical devices[235][2]	2001	No recommendation
RKI	Hygiene requirements for reprocessing of medical devices[236]	2012	**Not recommended**
SGNA	Standards of Infection Control in Reprocessing of Flexible Gastrointestinal Endoscopes[237]	2013	No recommendation
WGO/OMED	WGO/OMED Practice Guideline Endoscope Disinfection[238]	2005	**Recommended**
WGO/WEO	Endoscope disinfection - a resource-sensitive approach[239]	2011	No recommendation

[1]These guidelines were updated in 2008 by guidelines[231]; [2]These guidelines were updated in 2012 by guidelines[236]. AORN: Association of periOperative Registered Nurses; APIC: Association for Professionals in Infection Control and Epidemiology; APSIC: Asia Pacific Society of Infection Control; ASGE: American Society for Gastrointestinal Endoscopy; BSG: British Society of Gastroenterology; CDC: Centers for Disease Control and Prevention; ESGE: European Society of Gastrointestinal Endoscopy; ESGENA: European Society of Gastroenterology and Endoscopy Nurses and Associates; HPS: Health Protection Scotland; JGETS: Japanese Gastroenterological Endoscopy Technicians Society; OMED: Organisation Mondiale d'Endoscopie Digestive/World Organization for Digestive Endoscopy; RKI: Robert Koch Institute; SGNA: Society of Gastroenterology Nurses and Associates, Inc; WEO: World Endoscopy Organization (former OMED); WGO: World Gastroenterology Organisation.

tor[203], presence of biofilm on undamaged channels[205], an endoscope defect[203], delayed pre-wash resulting in drying of the gastroscope[207], and incorrect connectors joining the bronchoscope suction channel to the STERIS SYSTEM 1 processor[206]. Strict adherence to infection control guidelines for reprocessing endoscopes is therefore the key element for prevention of endoscope-associated outbreaks[203].

CLINICAL SIDE EFFECTS OF PERACETIC ACID

The potential health risks associated with all high-level disinfectants are considered to be serious, though little is known about the risks to humans, especially employees, from glutaraldehyde alternatives[208,209]. Gutterman *et al*[209] identified only eight studies "which reported numerous adverse outcomes to healthcare personnel associated with endoscope reprocessing", including one case report with asthma for workers using a peracetic acid and hydrogen peroxide based product. The most commonly-reported side effect of peracetic acid in patients is a form of colitis, previously known as pseudolipomatosis[210], which is commonly induced by hydrogen peroxide and peracetic acid but occasionally also by glutaraldehyde[211]. The colitis is often self-limiting but sometimes requires medical treatment. The frequency of colitis caused by peracetic

Table 5 Effects and possible outcomes of peracetic acid use for cleaning flexible endoscopes

Characteristic, reason for cleaning step	Effect of peracetic acid	Possible outcome, compared with classical cleaning
Removal of biofilm	Variable[1]	Insufficient removal of biofilm
Fixation of biofilm	Possible[1]	Fixation of biofilm to variable degrees
Removal of dried blood	Partial removal[1]	Insufficient removal of dried blood
Fixation of dried blood	Very likely	Fixation of dried blood to variable degrees
Fixation of brain tissue	Very likely	Strong fixation of nerve tissue, including prions
Adaptation of microorganisms surviving the cleaning step	Likely, especially in gram-negative bacteria	Insufficient efficacy of disinfection step, persistence of pathogens, beginning of biofilm formation
Cross-resistance to other biocidal compounds as a result of exposure to sublethal peracetic acid concentrations	Possible	Insufficient efficacy of disinfection step, persistence of pathogens, beginning of biofilm formation

[1]Depending on the formulation.

acid might be underestimated[212]. An overview of all reported cases is summarized in Table 3.

REVIEW OF NATIONAL AND INTERNATIONAL GUIDELINES

An overview of 17 guidelines from 14 different institutions is given in Table 4. Most institutions make no statement on the suitability of peracetic acid for cleaning flexible endoscopes, but there seems to be a recent trend in a few institutions to either skip their earlier recommendations of peracetic acid (ESGE/ESGNA and WGO/WEO) or to state that it is not suitable for cleaning (RKI).

CONCLUSION

Few national and international guidelines highlight the need for the cleaning of flexible endoscopes to be carried out using formulations without any fixation potential, but use of peracetic acid for cleaning is discouraged. Some peracetic acid-based formulations have some cleaning capacity. However, we found no conclusive evidence to suggest that the cleaning capacity of any peracetic acid-based formulation was as good as that of detergent-based cleaning agents without biocidal agents. Different peracetic acid-based formulations have been shown to enhance surface fixation of dried blood (all tested formulations), biofilm (some tested formulations) and brain tissue (all tested formulations). Fixed blood and biofilm are likely to impair the efficacy of the disinfection step, given that peracetic acid is known to lose its antimicrobial activity in the presence of various types of organic load. Fixed biofilm will reduce the susceptibility of microorganisms present in the biofilm, making it more difficult to achieve the required log-reduction during the disinfection phase. Even if the bacteria within a biofilm are killed by a disinfectant, microorganisms are likely to adhere to any residual biofilm structure within the endoscope more easily during the next endoscopic procedure.

Table 6 Practical tips to ensure optimal cleaning of flexible endoscopes

Clinical practice tip	Major advantage	Ref.
Clean promptly after use	No drying of organic material such as blood	[77,207]
Follow the instructions of the endoscope manufacturer as closely as possible (*e.g.*, type of brush or cleaning adapter)	Optimum cleaning of an entire channel	
Prefer washer disinfectors with a monitoring system indicating channel blockage	A blocked channel cannot be cleaned adequately and is immediately identified; targeted brush cleaning may be necessary	
Do not switch off the monitoring system for detection of blocked channels	Channels may be blocked and inadequately cleaned; personnel may not detect blocked channels with all possible implications for patient safety	
Support by gastroenterologist	It is strongly recommended that the clinician fully understands the cleaning and disinfection steps and does not inhibit his or her staff's ability to perform them correctly	[240]
Allow external audits by local health authorities on the quality of processing including cleaning	Implementation of guidelines may be more successful if the local health authorities visit the endoscopy units and compare current practices with the relevant guidelines. This effect seems to be more easily achieved in in-patient rather than in out-patient endoscopy units	[241-243]

Published research suggests that peracetic acid-based agents are not suitable for use in the cleaning step during the processing of flexible endoscopes (Table 5). However, some practical tips may help to improve the quality of the cleaning step (Table 6). This review highlights that protocols for processing flexible endoscopes should be evidence-based, rather than being based on convenience[213].

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