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In-hospital evaluation of orthophthalaldehyde as a high level disinfectant for flexible endoscopes

M. J. Alfa and D. L. Sitter

Department of Microbiology, St. Boniface General Hospital, 409 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6

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Summary: One hundred endoscopes used for bronchoscopy (30), gastroscopy (35) or colonoscopy (35) were studied to determine the efficiency of a new high level disinfectant, orthophthalaldehyde (OPA). Manual cleaning was the method studied since this would be the least effective and thereby provide the greatest challenge to the 0.5% (w/v) OPA solution. The OPA was convenient and easy to use since it did not have irritating vapours and as it is used directly, does not require dilution. Our study demonstrated that the OPA was stable for up to 14 days despite repeated re-use. The cleaning/disinfection procedure could achieve a $\geq 5 \text{ LOG}_{10}$ reduction in bacterial load. This in-hospital evaluation supports the conclusion that OPA is an effective choice as a high level disinfectant for flexible endoscopes.

Keywords: Orthophthalaldehyde; disinfection; flexible endoscopes.

Introduction

Endoscopy has become a commonplace procedure in the medical field. By far the largest number of such procedures is performed on the respiratory tract (bronchoscopies) or the gastrointestinal tract (gastroscopies or colonoscopies). When endoscopes are used in humans, the instrument is exposed to two broad categories of microorganisms (including bacteria, fungi, viruses and parasites): those that are part of the normal flora, and those that are not normal flora but are primary pathogens. It has been argued that the presence of normal flora in the upper respiratory tract and the gastrointestinal tract precludes the need to use a sterile endoscope. However, subsequent reports have clearly indicated that serious, sometimes life-threatening, infections can be caused by both of the aforementioned groups of organisms. Patient-to-patient transmission of Salmonella spp.,^{1,2} Helicobacter pylori,³ Pseudomonas aeruginosa,^{4,5} Bacillus spp.,⁶ Serratia spp.,^{7,8} and hepatitis B virus (HBV)⁹ have been documented. By far the most serious risk of infection occurs when endoscopes are used to access organs such as the pancreas and the gallbladder that are normally sterile. The duodenoscope used for endoscopic retrograde cholangio-pancreatography

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(ERCP) passes through the duodenum and instruments are passed through the endoscope into the biliary tree. In almost all cases, nosocomial infections occurring as a consequence of ERCP, were traced to inadequate disinfection of the endoscopes used,⁵ or trauma to tissue that resulted in dissemination of bacteria from the colonizing flora.¹⁰ Indeed, Allen *et al.*⁵ demonstrated that, when the duodenoscopes that were used had inadvertently been contaminated with *P. aeruginosa* approximately one-third of the patients developed *P. aeruginosa* biliary tract infections.

Because the endoscope passes over mucous membranes, high level disinfection rather than sterilization is acceptable. The choice of high level disinfectant is of primary concern and detailed guidelines have been formulated to facilitate this decision-making process.^{11,12} The Working Party of the British Society of Gastroenterology¹² has recommended alkaline glutaraldehyde (2%) and Gigasept (10%) (Butan 1-4 Dial/2,5 dimethoxy tetra-hydrofuran and formaldehyde) as effective antibacterial and antiviral agents that would adequately eradicate both human immunodefiency virus (HIV) and HBV as well as vegetative bacteria.

The need for activation of 2% glutaraldehyde makes it a labour-intensive disinfectant, and the irritating fumes associated with formaldehyde and glutaraldehyde make these disinfectants difficult to work with.¹³ It is apparent that there is a need for an effective high-level disinfectant that requires less handling, has a long shelf-life, and has fewer toxic effects than aldehydes.

In-hospital evaluations of disinfectants have been done for 'targeted' populations.¹⁴⁻¹⁶ However, in-hospital evaluations of the effectiveness of high level disinfectants for a broad range of instruments that include bronchoscopes, gastroscopes and colonoscopes are limited. This study was aimed at determining if a new high level disinfectant based on the active ingredient, orthophthalaldchyde (OPA) (Johnson and Johnson Inc.), is effective as a disinfectant for these three major types of endoscopes.

Materials and methods

Orthophthalaldehyde disinfectant

The OPA solution (0.5% w/v) was prepared by Johnson and Johnson Inc. It was stored at room temperature and was used directly as a 0.5% solution. Johnson and Johnson Inc. have conducted in vitro tests¹⁷ and their product label efficacy claims state that OPA (0.5%) used at 20°C for 5 min is bactericidal for: *Staphylococcus aureus*, *Salmonella choleraesuis*, *P. aeruginosa*; fungicidal for *Trichophyton mentagrophytes*; tuberculocidal for *Mycobacterium bovis* BCG; virucidal for: poliovirus Type 1, influenza virus (Hong Kong strain), herpes simplex virus type 1, herpes simplex virus type 2, adenovirus, rhinovirus type 42 and HIV-1; and sporocidal for: *Bacillus subtilis* and *Clostridium sporogenes*.

Specimen collection

This 'in hospital' evaluation of OPA as a high-level disinfectant of flexible endoscopes was performed at St. Boniface General Hospital (Winnipeg, Manitoba).

A total of 100 endoscopes was assessed after cleaning and disinfection with OPA to determine if there were any residual bacteria, viruses, parasites or fungi. There were 30 bronchoscopes, 35 gastroscopes and 35 colonoscopes evaluated. There were 10 from each group that were also tested immediately after use, i.e. prior to washing or disinfecting, to determine the load of organisms on endoscopes that were used for the three sites indicated. The level of bacteria and fungi was quantitatively determined by preparing serial 1:100 dilutions of the sample and spread-plating $100 \,\mu$ l of each dilution onto blood agar, chocolate agar and MacConkey agar. Detection of viruses, parasites and Clostridium difficile toxin was done using qualitative measurements. The endoscopes were cleaned using protozyme (Ruhof Corp., Valley Stream, N.Y.), disinfected for 5 min at room temperature ($\geq 20^{\circ}$ C) with OPA, and then the residual load of microorganisms was monitored by sampling the suction channel. Each sample consisted of approximately 10 ml of antibiotic-free tissue culture medium (RPMI base supplemented with glutamate, and 10% fetal bovine serum) that was drawn through the suction channel. The 10% serum in the tissue culture medium was known to effectively inactivate residual trace amounts of OPA or detergent, thereby ensuring optimal conditions to detect microorganisms. Each 10-ml sample was aliquoted as follows: 1 ml-viral transport media for viral culture; 2 ml-sterile tube for HIV and HBV ELISA tests; 2 ml-mycobacterial culture; 1 ml-in SAF for parasitology; 2 ml-for routine mycological and bacteriological culture and the remaining 2 ml was stored at -70° C. Organisms were identified using standard microbiological procedures. Identification of the organisms to the species level was done when possible using Microscan panels (Baxter Canlab Ltd) or API 20C strips (Sherwood Medical, Plainview, New York).

Gas chromatography assay of orthophthalaldehyde in solution (Direct Injection Method)¹⁸

The gas chromatography (GC) was performed on a DB-1 column in a varian 3700 GLC using helium as the carrier gas. The injector temperature was 250°C and the detector temperature was 280°C. The run time was 8 min.

Aqueous orthophthalaldehyde was mixed with an internal standard (piperonal) solution in methanol and analysed by gas chromatography. Peak areas were used to calculate the concentration of orthophthalaldehyde in solution.

Viral culture

Viral cultures were performed by routine tissue culture procedures to detect adenoviruses, enteroviruses, herpes simplex virus and myxoviruses. The

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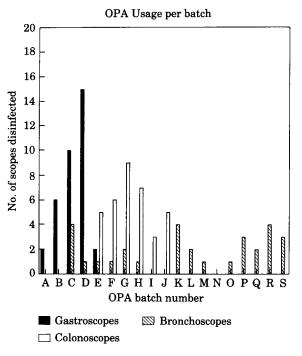


Figure 1. OPA usage per batch.

ELISA based HIV Ag1 kit (Abbott Laboratories, Abbott Park, IL) was used to detect HIV-1 antigens in the samples drawn through the endoscopes. The 'Auszyme' monoclonal antibody for HBsAg was used in an ELISA-based assay to detect surface antigen of HBV.

Results

The majority of the colonoscopies and gastroscopies were performed for non-infectious disease reasons, whereas the majority of the bronchoscopies were performed to facilitate diagnosis of infectious processes.

A total of 19 batches of OPA were utilized for 14 days each. The temperature of the OPA solution in the trays was measured and all endoscopes were disinfected at $\geq 20^{\circ}$ C. The concentration of orthophthalaldehyde was > 0.45% of all samples tested. Indeed, there was a slight increase from a concentration on day 1 of 0.55% to 0.59% by day 14. The maximum number of endoscopes disinfected in any one batch of OPA was 16 (Figure 1).

Samples were drawn from 10 bronchoscopes, 10 gastroscopes and 10 colonoscopes after in-hospital use, but before cleaning or disinfecting. This served as baseline data regarding the level of bacterial contamination of these groups of endoscopes. The average load of all types of microorganisms

Disinfection of endoscopes

Organism	Mean average cfu ml ⁻¹	No. times isolated	No. bronchoscopes detected in
Gram-positive cocci		· ·	
Micrococcus spp.	2.00×10^{3}	3	2
Staph. aureus	9.68×10^{2}	4	4
Staph. epidermidis	$2 \cdot 10 \times 10^{2}$	4	4
Staph. haemolyticus	9.00×10^{1}	1	1
Strep. bovis	9.00×10^{4}	1	1
Strep. Group F	4.00×10^{1}	1	1
Strep. intermedius	1.00×10^{1}	1	1
Strep. mitis	4.50×10^{1}	2	2
Strep. pneumoniae	7.50×10^{1}	2 2 1	2 2
Strep. salivarius	1.00×10^{1}	1	1
Strep. sanguis	5.07×10^{3}	2	2
Viridans streptococci	1.00×10^{1}	1	1
Gram-positive rods		-	-
Diphtheroids	5.04×10^{4}	5	4
Gram-negative cocci		·	
Neisseria spp.	9.70×10^{1}	3	2
Gram-negative rods		·	-
Acinetobacter anitratus	1.80×10^{4}	1	1
Haemophilus para-		•	-
influenzae	6.00×10^{1}	1	1
Klebsiella oxytoca	1.00×10^{1}	1	Î
Moraxella sp.	1.00×10^{1}	1	î
Fungi		1	
Candida albicans	1.0×10^{4}	2	2
Sacch. cerevisiae	1.0×10^{1}	1	1
Exophiala sp.	1.0×10^{1}	1	1

Table I. Bacterial and fungal counts (cfu ml^{-1}) from dirty bronchoscopes*

* No viruses were detected.

on the bronchoscopes before cleaning/disinfection was 6.4×10^4 cfu ml⁻¹. A breakdown of the various groups of bacteria isolated from bronchoscopes is shown in Table I. Streptococci were detected in 8/10 of the dirty bronchoscopes. This reflects the streptococci that are normal upper respiratory flora. The pattern of isolates from bronchoscopes was similar to the baseline data for gastroscopes (Table II). However, it differs in that the average load of microorganisms was higher at 1.7×10^5 cfu ml⁻¹ and the occurrence of isolation of Gram-positive rods was greater (9/10 for gastroscopes vs 4/10 for bronchoscopes). This probably reflects the higher load of diphtheroids in the gastrointestinal tract. The numbers and distribution of microorganisms isolated from the colonoscopes before cleaning/disinfection reflects a very different type of contaminating flora (Table III). The average load of bacteria was $5 \cdot 2 \times 10^5$ cfu ml⁻¹ and was due to the higher concentration of microorganisms in the colon compared to the upper gastrointestinal tract or the bronchi. The predominant bacteria were Gram-negative bacilli (9/10 colonoscopes) and Gram-positive cocci (9/10 colonoscopes). Although Blastocystis hominis was found in two

Organism	Mean cfu ml ⁻¹	No. of times isolated	No. of gastroscopes detected in	
Gram-positive cocci				
Micrococcus spp.	1.01×10^{3}	3	2	
Staph. aureus	2.00×10^{1}	1	2	
Staph. epidermidis	5.40×10^{2}	2	2	
Staph. haemolyticus	9.00×10^{10}	2 4	2 2 1	
Staph. warneri	2.00×10^{1}	1	1	
Strep. bovis	5.70×10^{3}	3	3	
Strep. intermedius	1.10×10^{2}	1	1	
Strep. mitis	3.36×10^{4}	5		
Strep. morbillorum	6.00×10^{5}	1	3 1	
Strep. salivarius	1.00×10^{3}	2	2	
Strep. sanguis	3.00×10^{10}	1	1	
Viridans streptococci	6.29×10^{4}	5	4	
Gram-positive rods				
Diphtheroids	5·14 × 10 ⁵	15	8	
Lactobacillus sp.	1.40×10^{4}	1	1	
Gram-negative cocci				
Neisseria spp.	3·34 × 10⁴	3	3	
Gram-negative rods				
Enterobacter cloacae	5.05×10^{3}	1	1	
Esch. coli	1.00×10^{1}	1	1	
Pseud. aeruginosa	9.00×10^{2}	1	1	
Pseudomonas spp.	5.60×10^{2}	1	1	
Fungi			_	
Candida albicans	1.0×10^{4}	2	2	
Candida tropicalis	3.8×10^{4}	1	$\overline{1}$	
Candida glabrata	2.0×10^{3}	1	1	
Penicillium sp.	1.0×10^{1}	1	1	

Table II. Bacterial and fungal counts (cfu ml^{-1}) from dirty gastroscopes*

* No viruses or parasites were detected.

colonoscopes, and various fungi were detected, none of the 30 dirty endoscopes sampled contained detectable levels of virus (Table IV).

Each of the 10 dirty bronchoscopes, gastroscopes and colonoscopes was washed and disinfected with OPA. The disinfection consisted of a 5 min soak in OPA at room temperature. Also, a further series of 20 bronchoscopes, 25 gastroscopes, and 25 colonoscopes were washed and disinfected after routine in-hospital use. The average temperature of the OPA solution used to disinfect the 100 endoscopes was $24 \cdot 1^{\circ}C$ (± 0.92 sp). The disinfected endoscopes were then sampled according to the method described to determine if there were any residual microorganisms. None of the 100 disinfected endoscopes had residual bacteria, fungi, parasites or viruses. The overall summary of the effectiveness of OPA as a disinfectant is presented in Table IV. This demonstrates that even high levels of bacteria $(1 \times 10^{6} \text{ cfu ml}^{-1})$ were eliminated to below the limit of detection (10 cfu ml $^{-1}$); this represented a $\geq 5 \log_{10}$ decrease in bacterial counts.

All samples taken from the endoscopes were examined by Gram's stain. It is of interest to note that, despite cleaning and disinfecting, some of the

Disinfection of endoscopes

Organism	Mean cfu ml ⁻¹	No. times isolated	No. of colonoscopes detected in
Gram-positive cocci			
Enterococcus faecalis	5.00×10^{3}	1	1
Enterococcus faecium	6.50×10^{4}	2	2
Micrococcus spp.	1.00×10^{1}	1	1
Staph. aureus	1.00×10^{3}	1	1
Staph. haemolyticus	2.00×10^{3}	3	3
Staph. simulans	3.00×10^{1}	1	1
Strep. Group G	2.00×10^{3}	1	1
Strep. intermedius	2.50×10^{5}	2	2
Strep. mitis	2.00×10^{3}	1	1
Strep. salivarius	4.25×10^{6}	2	2
Viridans streptococci	1.12×10^{5}	6	6
Streptococcus sp.	1.00×10^{1}	1	1
(beta haemolytic)	1.02×10^{3}	2	2
Streptococcus sp.	1.02×10^{-5}	Z	2
(non-haemolytic)			
Gram positive rods	2.01×10^{5}	10	-
Diphtheroids	2.91×10^{5} 1.00×10^{5}	10	5
Lactobacillus spp.	$1.00 \times 10^{\circ}$	1	1
Gram-negative cocci	7.00×1.01	1	1
Neisseria spp.	7.00×10^{1}	1	1
Gram-negative rods			
Citrobacter	1 00 106		
amalonaticus	1.00×10^{6}	1	1
Citrobacter freundii	1.69×10^{3}	3	2
Esch. coli	1.64×10^{6}	9	6
Hafnia alvei	3.00×10^{3}	1	1
Klebsiella oxytoca	5.00×10^{2}	1	1
Klebsiella pneumoniae	1.07×10^{6}	3	3
Proteus penneri	2.00×10^{5}	1	1
Pseudomonas aeruginosa	9.00×10^{1}	1	1
Serratia marcescens	1.00×10^{2}	1	1
Fungi			
Candida albicans	1.0×10^{4}	6	6
Rhodotorula rubra	1.0×10^{1}	1	1
Candida glabrata	2.0×10^{3}	1	1
Aspergillus spp.	1.0×10^{1}	1	1
Wangiella spp.	2.0×10^{1}	1	1

Table III. Bacterial and fungal counts (cfu ml^{-1}) from dirty colonoscopes*

* In addition, Blastocystis hominis was detected from 2 colonoscopes; No viruses were detected.

stains revealed that bacteria were still present in the endoscopes. A similar pattern was seen for endoscopes disinfected with 2% glutaraldehyde. It is likely that these bacteria were dead, since no viable microorganisms were recovered in culture.

Discussion

This is the first in-hospital evaluation of the ability of OPA to eradicate microorganisms from endoscopes used for routine medical procedures. Our

Organism	Dirty	Disinfected
Bronchoscopes	n = 10	n=30
Gram-positive cocci	4.7×10^{3}	0*
Gram-positive bacilli	2.8×10^{5}	0
Gram-negative cocci	1.3×10^{2}	0
Gram-negative bacilli	6.0×10^{3}	0
Yeast	1.5×10^{2}	0
Other fungi	1.0×10^{1}	0
Parasites	ND‡	ND
Virus	Neg	Neg
Gastroscopes	n = 10	n = 35
Gram-positive cocci	1.6×10^{4}	0
Gram-positive bacilli	8.5×10^{5}	0
Gram-negative cocci	3.4×10^{4}	0
Gram-negative bacilli	1.9×10^{3}	0
Yeast	3.6×10^{4}	0
Other fungi	1.0×10^{1}	0
Parasites	ND	ND
Virus	Neg	Neg
Colonoscopes	n = 10	n = 35
Gram-positive cocci	2.7×10^{5}	0
Gram-positive bacilli	4.6×10^{5}	0
Gram-negative cocci	7.0×10^{1}	0
Gram-negative bacilli	1.0×10^{6}	0
Yeast	4.0×10^{2}	0
Other fungi	1.5×10^{1}	0
Parasites [†]	Pos	Neg
Virus†	Neg	Neg

Table IV. Overall summary of OPA effectiveness

* Limit of detection = 10 cfu ml⁻¹; \dagger Quantitation not done, only reported as positive or negative; $\ddagger ND = Not$ done for this site.

study demonstrated that the OPA solution was very stable over 14 days and that it effectively disinfected bronchoscopes, gastroscopes and colonoscopes.

Hanson et al.¹⁶ reported that re-use of 2% glutaraldehyde for 20 endoscopes reduced the concentration to 1% glutaraldehyde. They found that increased protein load due to serum significantly impaired the efficiency of 1% glutaraldehyde. Our chemical analysis of 19 separate batches of OPA over 14-day usage cycles indicated that the concentration of active ingredient remained >0.45% over the entire 14-day period, despite cumulative protein load from disinfecting up to 16 endoscopes (Figure 1). Although the OPA was stored in the disinfection 'tubs' that had lids, it is likely that some evaporation occurred. This would account for the gradual increase in OPA concentration noted.

The baseline data collected from 'dirty' endoscopes immediately after use indicated that the load of microorganisms ranged from 6×10^4 cfu ml⁻¹ for bronchoscopes (Table I) to 5×10^5 cfu ml⁻¹ for colonoscopes (Table III).

This difference is expected because of the different concentrations of normal flora in these body sites, which was less than the 10^9 cfu ml⁻¹ upper range that was reported by Dumon *et al.*¹⁹ The method of sampling accounts for this, since Dumon *et al.*¹⁹ reported the concentration of microorganisms isolated in the direct bronchial secretions, whereas, in this study the counts were determined from a 10-ml sample of sterile fluid that was drawn through the 'used' dirty endoscope. The types of pathogenic microorganisms isolated from this study were similar to those detected by Dumon *et al.*¹⁹ Of the ten 'dirty' bronchoscopes assessed, nine had bacteria typical of the normal flora of the upper respiratory tract and seven had potentially pathogenic bacteria isolated. None of the bronchoscopes in this study grew mycobacteria (Table IV). Since only 10 dirty bronchoscopes were evaluated and because of the low incidence of tuberculosis in our population, the likelihood of isolating mycobacteria was low.

The types and average load of organisms isolated from the gastroscopes reflected the normal flora of the upper respiratory tract and were similar to those reported by Hanson *et al.*²⁰ in patients with AIDS. We did not isolate any *H. pylori* or viral pathogens (Table IV). This again reflects the study population since the gastroscopies were performed almost exclusively for reasons other than infectious disease.

The higher concentration of bacteria found in endoscopes after colonoscopy (Table III) is expected due to the high concentration of bacteria in the bowel. The patients had fasted prior to the colonoscopy procedure; therefore, although the bacterial load was greater than seen for bronchoscopes and duodenoscopes, it was not as heavy as would be found in direct faecal material. The microorganisms isolated (Table III) reflected the bowel flora where Gram-negative rods are far more prevalent than in either the respiratory tract or the upper GI tract. It is of interest that 6/10 'dirty' colonoscopes were contaminated with Candida albicans (Table III). Indeed. the fungal load was greatest in colonoscopes. The only parasite detected was B. hominis (Table IV) from two of the 'dirty' colonoscopes. Since C. difficile can sporulate, it would be of great interest to determine if contamination of endoscopes could result in patient-to-patient transfer. A prospective study by McFarland, Surawicz & Stamm²¹ indicated that endoscopy was a risk factor for development of antibiotic-associated pseudomembranous colitis. Although we specifically looked for C. difficile toxin by a selective enrichment procedure, all 10 'dirty' colonoscopes and all of the 35 disinfected endoscopes were negative (Table IV). The small sample size of 10 dirty colonoscopes does not preclude the possible transmission of C. *difficile* spores and this area requires further evaluation.

Despite an adequate specimen collection, transport, and culture approach, no viruses were detected. These results were similar to those of Hanson *et al.*¹⁶ where, of the 68 bronchoscopes evaluated, none of the 'dirty' bronchoscopes grew any viruses. The negative viral cultures and antigen detection tests indicated that either there were no viable viruses or that the

amount was less than the limit of detection of the tests used. The baseline data demonstrated that, for colonoscopes, the average bacterial load was $5\cdot 2 \times 10^5$ cfu ml⁻¹. These highly contaminated colonoscopes were still effectively disinfected by OPA. This represented a >5 log₁₀ reduction in bacterial counts. This in-hospital evaluation demonstrated that a 5 min soak in OPA effectively eradicated microorganisms from bronchoscopes, gastroscopes and colonoscopes (Table IV). The washing process by itself can remove up to 10^3 organisms, thereby leaving fewer organisms for the disinfectant to kill. This does not detract from our conclusion on the effectiveness of OPA but rather emphasizes the need to combine good washing technique with any high level disinfectant to ensure maximal efficiency of the disinfectant.

The demand for endoscopy procedures has significantly increased over the last 10 years and is expected to continue increasing as new procedures are developed. Indeed, Scott²² estimated that, in England, during the 1990s, the annual demand for endoscopy could be 12/1000 population. Recent surveys indicate that cleaning and disinfection procedures may be inadequate in up to 30% of the centres surveyed.²³ This raises serious concerns regarding infection control. Contaminated endoscopes have been well documented as vectors of not only 'normal bacterial flora', but also primary pathogens or water-associated bacteria. Regardless of which disinfectant is utilized, the importance of adequate cleaning cannot be over-emphasized. Regardless of how effective a disinfectant is, if it cannot adequately penetrate the caked-on proteins, is inactivated by too much protein, or is diluted too much, it will be ineffective. The effect of dilution is particularly critical when endoscope washers are used. Care must be taken that adequate quality assurance is done to ensure that the concentration of disinfectant remains within the range of optimal activity. Felmingham et al.²⁴ reported that, even if adequate disinfection of endoscopes is achieved, if water is left in the endoscope channels, then the endoscopes will have high bacterial levels after sitting overnight. This was confirmed by Alfa and Sitter.²⁵

Ridgway¹³ indicated that a suitable disinfectant should be "microbiologically effective, rapid in action, not significantly affected by organic material, not damage the endoscope and not cause hypersensitivity in the users". With these parameters in mind, this study presents in-hospital data that demonstrate that OPA is an effective high level disinfectant for eradicating vegetative bacteria, fungi and parasites from bronchoscopes, gastroscopes and colonoscopes. Unlike many other high level disinfectants, there is no need to activate or dilute the OPA solution. These features, combined with stability over the 14-day usage cycle, make it an effective alternative choice.

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