Disinfection of upper gastrointestinal fibreoptic endoscopy equipment: an evaluation of a cetrimide chlorhexidine solution and glutaraldehyde

D. L. CARR-LOCKE AND P. CLAYTON

From the Endoscopy Unit, and Department of Microbiology, Leicester General Hospital, Leicester

SUMMARY   There is little information available on the bacteriological contamination of upper gastrointestinal fibreoptic endoscopes during routine use and the effects of 'disinfecting solutions'. A bacteriological evaluation was therefore made of cleaning an endoscope and its ancillary equipment with (1) water, (2) an aqueous solution of 1 % cetrimide with 0·1 % chlorhexidine, and (3) activated aqueous 2 % glutaraldehyde. All equipment, but particularly the endoscope itself, was found to be heavily contaminated after use with a wide variety of organisms of which 53 % were Gram positive. Cleaning the endoscope and ancillary equipment with water and the cetrimide/chlorhexidine solution alone or in combination was inadequate to produce disinfection but immersion in glutaraldehyde for two minutes consistently produced sterile cultures with our sampling technique. A rapid and simple method for disinfection of endoscopic equipment is therefore recommended and we think this is especially suitable for busy endoscopy units.

Diagnostic and therapeutic fibreoptic endoscopy is being increasingly performed in this country in the investigation and treatment of upper gastrointestinal disease. Many endoscopists, however, remain complacent about the need to disinfect upper gastrointestinal instruments between successive examinations despite advice from instrument manufacturers and previous reports (Axon *et al.*, 1974; Elson *et al.*, 1975; Raines *et al.*, 1975; Shull *et al.*, 1975; Tolon *et al.*, 1976; Dunkerley *et al.*, 1977; Noy *et al.*, 1977). Although the extreme situation of fatal septicaemia after endoscopy in granulopenic patients (Greene *et al.*, 1974) is comparatively rare, the risks of bacteraemia and cross-infection when successive patients are examined with the same instrument are high (Elson *et al.*, 1975; Raines *et al.*, 1975; Shull *et al.*, 1975). There is, therefore, a need for a rapid and safe method of endoscope disinfection. To our knowledge there have been only five reported studies of disinfection of upper gastrointestinal endoscopes (Axon *et al.*, 1974; Elson *et al.*, 1975; Tolon *et al.*, 1976; Dunkerley *et al.*, 1977; Noy *et al.*, 1977), four of which suggest that activated glutaraldehyde is an effective agent as has been shown with other instruments (Ross, 1966; Mitchell and Alder, 1975). Recommended procedures for disinfecting instru-

ments with glutaraldehyde have ranged from 'thorough irrigation' (Noy *et al.*, 1977) to immersion times of from 10 minutes (American Hospital Association, 1974; Axon *et al.*, 1974; Salmon, 1974; Elson *et al.*, 1975; Tolon *et al.*, 1976) to 20 minutes (Blumgart, 1975; KeyMed (personal communication)) and 30 minutes (Haglof, 1976), although it has been shown that vegecidal activity is present after only two minutes (Stonehill *et al.*, 1963; Borick *et al.*, 1964). Tuberculocidal and virucidal activity require 10 minutes' exposure (Stonehill *et al.*, 1963; Klein, 1963), but sporicidal activity requires at least three hours (Stonehill *et al.*, 1963; Lowbury *et al.*, 1975). As fibreoptic equipment contains special synthetic materials, few of the common sterilising procedures normally used in hospital practice are safe for use with endoscopic equipment (KeyMed, personal communication). Ethylene oxide gas sterilisation has been shown to be effective (Chang *et al.*, 1973; Axon *et al.*, 1974), but this method is still not widely available.

The need for further bacteriological studies in this area has been expressed (Schiller and Salmon, 1976) and we therefore decided to investigate the effects of cleaning an upper gastrointestinal endoscope and its standard ancillary equipment during routine use with (1) tapwater, (2) a solution of aqueous 1 % cetrimide and 0·1 % chlorhexidine commonly used in this

hospital for cleaning purposes, and (3) activated aqueous 2% glutaraldehyde (Cidex, Arbrook Products), made up according to manufacturer's instructions. The ultimate purpose of this study was to ensure that a short cleaning technique during an endoscopy session was effective and would still allow a large number of patients to be examined.

Methods

MATERIALS

All bacterial samples were collected and cleaning techniques evaluated during routine upper gastrointestinal endoscopy sessions at the Leicester General Hospital endoscopy unit between June and November 1976 and during October 1977. The instrument tested was the Olympus GIF-K with standard ancillary equipment. Some test items were inoculated directly onto horse blood agar plates—namely, KY jelly, local anaesthetic spray (1% lignocaine, Astra), dimethicone antifoaming solution, and mains tap-water. Some specimens were collected by flushing or washing the relevant piece of equipment with 10 ml sterile water and plating this immediately onto blood agar. The majority of samples, however, were collected by accurate swabbing of the item under test with a standard throat swab moistened with sterile water, the specimen then being plated out immediately onto blood agar. All specimen plates were then transported to the microbiology department, where they were incubated aerobically at 37°C for 24 hours. Plates were then inspected, colonies counted, and selected colonies taken for further identification by standard methods (Cowan and Steele, 1974). Inactivators were not used in the main study but it is known that washing for 30 seconds with sterile distilled water physically removes glutaraldehyde (Trent Regional Hospital Authority, Drug Information Centre, 1977, personal communication). In a subsequent study samples were collected in triplicate from the endoscope only and were inoculated separately onto blood agar and into Robertson's cooked meat medium, and also Brewer's medium to each of which had been added 1% sodium thiosulphate as an inactivator of glutaraldehyde (Rubbo and Gardner, 1965; Ross, 1966). Subcultures on blood agar from broth cultures were examined after overnight incubation at 37°C. With the exception of Genera *Micrococcus*, *Pseudomonas*, *Proteus*, and *Klebsiella*, all organisms were identified by a genus and species type.

Equipment was divided into five test groups, each undergoing a different test sequence of cleaning and attempted disinfection that was thought appropriate for that particular item. All steps in the sequence were performed on three separate occasions to provide a representative sample. The cetrimide/chlorhexidine solution was made freshly on each day of the investigation and glutaraldehyde was renewed weekly.

The five test groups were:

Group 1
This included items requiring basal cultures only to detect contamination. These were KY jelly, dimethicone, local anaesthetic spray, mains tap orifice, stainless steel sink outlet, mains tap water, endoscope camera, teaching attachment, and light source.

Group 2
This included the working surface of the endoscopy trolley and the internal surface of the endoscope storage cupboard. The latter underwent swabbing before the instruments were taken out of the cupboard for daily use, after the used instruments were returned, and after cleaning the cupboard with water, cetrimide/chlorhexidine, or glutaraldehyde. The trolley was sampled before and after use and then after cleaning with water, cetrimide/chlorhexidine, and glutaraldehyde and both items were swabbed on the morning after cleaning the previous day with either cetrimide/chlorhexidine or glutaraldehyde.

Group 3
This was the internal surface of the water supply bottle. This was sampled by rinsing with 10 ml sterile water before use, during an endoscopy session, after holding cetrimide/chlorhexidine for 30 minutes, and after holding glutaraldehyde for two, 10 and 20 minutes.

Group 4
This included the plastic mouthguard, biopsy forceps, suction tubing (sampled near the light source), and plastic cannula, which were swabbed before use, after use, and after cleaning with water, cetrimide/ chlorhexidine with two minutes' immersion, and glutaraldehyde with two and 10 minutes' immersion.

Group 5
The endoscope was sampled at 11 separate sites (Table), using the swabbing technique for all sites except the suction channel through which 10 ml sterile water was aspirated manually from the suction tubing connection point. Samples were taken before use, having been in the storage cupboard overnight, after use, and then after cleaning with water alone, or water followed by cetrimide/chlorhexidine, or water followed by glutaraldehyde with two, 10, and 30 minutes' immersion. The instrument was cleaned in a standard way throughout the test period with an immediate thorough water wash to remove debris

Table *Summary of results of cultures from 11 sites on endoscope GIF-K**

Endoscope	Before use	After use (all sites) (all profuse)	After water (all sites)	After cetrimide/ chlorhexidine (all sites)	After glutaraldehyde (all sites) 2 min	10 min	30 min
1 Bending section	Micrococcus spp. (Sm)	Strep. viridans	Strep. viridans (Pr)	Strep. viridans (Pr)		Strep. viridans (Sm)	
2 Distal forceps opening	Strep. viridans (Sc)	Pseudomonas spp.	Pseudomonas spp. (Pr)	Pseudomonas spp. (Pr)		Pseudomonas spp. (Sm)	
		Staph. coag. neg.	Staph. coag. neg. (Pr)	Staph. coag. neg. (Pr)			Staph. coag. neg. (Sc)
		E. coli	E. coli (Pr)	E. coli (Sc)		E. coli (Sc)	E. coli (Sc)
3 Objective lens	Staph. coag. neg. (Sc)	Proteus spp.	Proteus spp. (Pr)	Proteus spp. (Pr)			
4 Insertion tube, 20 cm	Strep. viridans (Sc)	N. catarrhalis	N. catarrhalis (Pr)				
		Staph. coag. pos.		Staph. coag. pos. (Sm)			
		Micrococcus spp.	Micrococcus spp. (Sm)			Micrococcus spp. (Sm)	
5 Insertion tube, 40 cm	Staph. coag. neg. (Mo)	B. haem. strep.					
		Klebsiella spp.		Klebsiella spp. (Pr)			
6 Insertion tube, 1 m	—	Corynebacterium hofmannii			—		
		B. subtilis	B. subtilis (Sc)	B. subtilis (Sc)			
7 Forceps valve opening	—	Acinetobacter lwoffii					
8 Forceps valve shelf	Pseudomonas spp. (Pr) E. coli (Sc)	C. albicans					
9 Suction connector	E. coli (Pr)						
10 Water connector	Pseudomonas spp. (Sm)						
11 Suction channel	Pseudomonas spp. (Pr) Str. viridans (Pr) Proteus spp. (Sc)						
No.* (%) sterile cultures	20/33 (61%)	21/165 (13%)	14/33 (42%)	12/33 (36%)	33/33 (100%)	23/27 (85%)	25/27 (93%)

*Colony counts are expressed as profuse (Pr), moderate (Mo), small (Sm), or scanty (Sc). See text for details.

from the outside of the insertion tube using an un-gloved hand and a clean paper towel. Adequate water was aspirated through the suction channel to wash out all debris and the special cleaning adaptor for insertion into the biopsy valve housing was used each time. The endoscope was then immersed in the test solution up to the 1 m mark on the insertion tube and the suction channel filled with the appropriate cleaning fluid under test (Figure). The endoscope was then rewashed in tap water and water aspirated to wash out cleaning fluid followed by air to empty the suction channel. It was finally dried completely with a sterile paper towel.

Results

Our initial cultures had shown that the cetrimide/chlorhexidine solution was sterile when inoculated into double strength Brewer's medium. Contact cultures of the hands of endoscopy staff grew *Staphylococcus coagulase negative* (*Staph. coag. neg.*) only. The results in each group are expressed as one of four possible categories according to colony counts on each blood agar plate inspected. These are profuse growth (greater than 200 colonies/plate), moderate growth (50 to 200 colonies/plate), small growth (between 10 and 50 colonies/plate), and scanty growth (less than 10 colonies per plate). A total of 626 plates were examined during the study period. The culture results presented here are summarised but full details can be provided on request.

GROUP 1
Cultures of KY jelly, dimethicone, local anaesthetic spray, mains tap water, and the mains tap orifice were sterile. Those from the sink outlet grew profuse *Pseudomonas spp.* and a small growth of *Bacillus subtilis*, *Staph. coag. neg.*, and *Strep. viridans*. The camera grew small numbers of *Staph. coag. pos.* and *B. subtilis*, the teaching attachment scanty *Staph. coag. neg.*, and the light source *Staph. coag. neg.* and *B. subtilis*.

GROUP 2
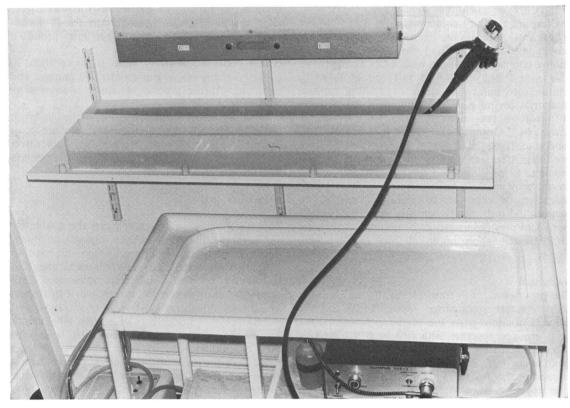Before use at the beginning of an endoscopy session samples from the endoscopy trolley grew *Staph. coag.*

Figure   *Olympus GIF-K immersed in cleaning fluid and attached to light source.*

neg., *Corynebacterium hofmannii*, *B. subtilis*, and *Acinetobacter lwoffii* in small numbers and the storage cupboard grew profuse *B. subtilis* and scanty *Staph. coag. neg.* After use, nine of 18 cultures remained sterile but there were larger numbers of the same organisms with the addition of *Strep. viridans* and *E. coli*. Water cleansing had a slight effect in reducing colony numbers, but only three of six cultures were sterile. After cetrimide/chlorhexidine there was no further change, but after cleaning with glutaraldehyde five of six cultures were sterile and there was only a scanty growth of *B. subtilis* in the one contaminated plate. Sampling both items on the morning after cleaning with either cetrimide/chlorhexidine or glutaraldehyde produced five out of six sterile cultures and six out of six sterile cultures respectively.

GROUP 3
The water supply bottle was contaminated by small numbers of *Neisseria catarrhalis* and *Acinetobacter lwoffii* and scanty *B. subtilis* before use in two out of three cultures. During an endoscopy session all samples grew profuse *Pseudomonas spp.* and small

numbers of *A. lwoffii* and *Staph. coag. neg.* After holding cetrimide/chlorhexidine for up to 30 minutes scanty *Pseudomonas spp.* were still recoverable in one out of three cultures. Two minutes and 10 minutes glutaraldehyde, however, produced scanty *B. subtilis* in one out of three plates, but no growth after 20 minutes' contact.

GROUP 4
Ten of 12 samples from the mouthguard, biopsy forceps, suction tubing, and cannula were sterile before use with only a small growth of *Strep. viridans* and *Micrococcus spp.* and scanty *Staph. coag. neg.* After use only 18 of 48 samples produced no growth, while the contaminated samples grew profuse *Strep. viridans*, *N. catarrhalis*, *Staph. coag. neg.*, *Micrococcus spp.*, β haemolytic strep. (not group A), *Staph. coag. pos.*, *Pseudomonas spp.*, *Proteus spp.*, *E. coli*, and *Alcaligenes faecalis*, the heaviest growth coming from the suction tubing. Water cleaning did not alter the type or number of colonies and cetrimide/chlorhexidine, while reducing the overall colony count, did not significantly increase the proportion of sterile plates. After two minutes

glutaraldehyde all cultures were sterile.

Cleaning ancillary equipment with water did not significantly change the bacterial population but cetrimide/chlorhexidine had some effect in reducing colony counts. Overall the number of sterile plates rose from 27 out of 69 (39%) to 10 out of 21 (48%) and the total number of growths in each of the four categories for all pieces of equipment fell from 33 (13 profuse, two moderate, eight small, and 10 scanty, 64% Gram positive) to 11 (one profuse, two moderate, three small, and five scanty, 70% Gram positive). After exposure to glutaraldehyde for two minutes, 19 of 21 plates (90%) were sterile with a scanty growth of *B. subtilis* in two cultures. Prolongation of exposure to glutaraldehyde to 10 minutes did not improve on this with 11 out of 15 sterile cultures (73%) but at 20 minutes in the case of the water supply bottle all three cultures were sterile.

GROUP 5

The result of cultures from 11 specified sites on the endoscope are summarised in the Table and the cultures obtained after use of the instrument have been grouped together, although each sample was taken immediately before exposure to the cleaning fluid under test. Before use 61% of cultures were sterile but this fell to 13% after use and, taking all sites together, showed heavy contamination with 51% Gram negative organisms. Of the 165 culture plates taken after use 90 plates were contaminated with *Strep. viridans* (55%), 50 with *Pseudomonas spp.* (30%), 35 with *Staph. coag. neg.* (20%), 22 with *E. coli* (13%), 22 with *Proteus spp.* (13%), 16 with *N. catarrhalis* (10%), eight with *Staph. coag. pos.* (5%), seven with *Micrococcus spp.* (4%), and four with *B. haemolytic strep.* (3·6%) in counts greater than 50 colonies per plate. Washing the instrument with tap water produced 14 out of 33 (42%) sterile cultures and the organisms recovered in more than 50 colonies/plate were *Pseudomonas spp.* in eight (24%), *Strep. viridans* in seven (22%), *N. catarrhalis* in three (9%), *Staph. coag. neg.* in three (9%), and *E. coli* in two (6%). This changed the percentage of Gram negative organisms to 67%, but, if all colonies were taken into account, this was 52% and thus much the same as before washing.

Cleaning with cetrimide/chlorhexidine produced 12 out of 33 sterile cultures (36%) and the organisms still recovered in more than 50 colonies per plate were *Proteus spp.* in six (18%), *Pseudomonas spp.* in six (18%), *Klebsiella spp.* in two (6%), *E. coli* in two (6%), *Staph. coag. neg.* in one (3%), and *Strep. viridans* in one (3%). This changed the percentage of Gram negative organisms to 89%, but if all colonies were taken into account this was 70%. Two

minutes' immersion in glutaraldehyde was highly effective with 33 out of 33 sterile cultures. Prolongation of disinfection to 10 and 30 minutes produced sterile cultures in 23 out of 27 (85%) and 25 out of 27 (93%) plates respectively with no more than 20 colonies of any one organism after 10 minutes and only one profuse growth of *Strep. viridans* after 30 minutes.

In the subsidiary study cultures from the two broth media containing 1% sodium thiosulphate gave results identical with those obtained from direct blood agar inoculation before and after disinfection with 2% glutaraldehyde.

Discussion

This study has set out to investigate the qualitative and semi-quantitative changes in saprophytic organisms on endoscopic equipment when subjected to cleaning with water, cetrimide/chlorhexidine, and glutaraldehyde. The disinfection times used have previously been shown to be vegicidal with respect to aqueous chlorhexidine (Davies *et al.*, 1954) and glutaraldehyde (Stonehill *et al.*, 1963). No attempt was made to assess virucidal, fungicidal, tuberculocidal, or sporicidal activity.

We have not found the widespread contamination of the endoscope itself with *Pseudomonas spp.* after storage as reported by Axon *et al.* (1974) and Tolon *et al.* (1976) apart from the suction channel and shelf below the biopsy valve. We attribute out findings to thorough drying of the instrument before it is replaced in the storage cupboard overnight as this seems to be independent of which disinfection procedure has been used. The two contaminated sites are difficult areas to dry after use.

It is clear that nearly all endoscopic equipment used in the upper gastrointestinal tract becomes heavily contaminated with a wide variety of Gram positive and negative organisms. If all cultures are taken into account the proportions of these are 53% and 47% respectively.

Of the ancillary equipment we have found that the mouthguard, biopsy forceps, water supply bottle, and endoscopy trolley become significantly contaminated during use but the cannula and cupboard less so. Suction tubing, not surprisingly, shows the heaviest contamination. Attempted disinfection with cetrimide/chlorhexidine was always inadequate even after 30 minutes' immersion in the case of the water supply bottle. There was little difference in contamination of the storage cupboard and endoscopy trolley samples on the morning after cleaning with either cetrimide/chlorhexidine or glutaraldehyde, but only the latter produced completely sterile cultures. Although the cannula in this study was that

designed for use with a panendoscope our results would probably apply also to the cannula used for endoscopic retrograde cholangiopancreatography passed down the suction channel of a duodenoscope. We recovered only insignificant colonies in three of 12 tests, however, and these were cleared by cetrimide/chlorhexidine and glutaraldehyde with two minutes' immersion in each case. We should add that ERCP cannulae can be sterilised by steam autoclaving and chemical disinfection should therefore be only a second-best alternative.

The most important findings relate to the endoscope itself, which showed heavy contamination after use with a wide variety of organisms. Cleaning with water and cetrimide/chlorhexidine was ineffective in eradicating the predominant organisms but two minutes' immersion in glutaraldehyde produced 100% sterile cultures.

It seems likely that faults in our swabbing technique in some instances led to false negative culture results, as evidenced particularly by sterile cultures after use of instruments, and false positive results such as the late apparent contamination after prolonged exposure to glutaraldehyde. Nevertheless, we feel that the numbers of cultures taken overcome these errors and qualitative and semi-quantitative changes in bacterial populations give a valid assessment of the cleaning techniques used. Adequate inactivation of glutaraldehyde by washing with water has been confirmed by the use of the specific inhibitor in the subsidiary study which gave identical results.

The cleaning and disinfecting method that we have now adopted as a result of this study, and performed both before an endoscopy list and after each examination, is that outlined above and is based on the manufacturer's instruction manual (KeyMed, 1977, personal communication). It must be stressed that thorough washing before disinfection is mandatory to preserve the life of the instrument and allow the agent to disinfect the most contaminated areas (Ross, 1966; KeyMed, 1977). The endoscope is kept horizontal during its two minutes' immersion in glutaraldehyde (Figure), as we feel that vertical immersion as previously described (Axon *et al.*, 1974) may allow entry of fluid into distal parts of the endoscope by the hydrostatic pressure of the column of fluid through small and inapparent breaks in the outer casing, especially that of the flexible bending section (KeyMed, 1977). This may be the reason for one previous report of damage after glutaraldehyde disinfection (Dunkerley and Mitchell, 1976), but its general safety with fibreoptic instruments and lens systems has been widely accepted (Stonehill *et al.*, 1963; Salmon, 1974; American Hospital Association, 1974; KeyMed, 1977). All equipment is washed with

water to remove glutaraldehyde and then thoroughly dried with a sterile paper towel. The adaptor designed to wash out the biopsy valve housing and upper suction channel is used at the beginning and end of the session only in combination with a cleaning brush, as is replacement of water in the water supply bottle by glutaraldehyde for two minutes to disinfect the water channel. Internal and external drying of all equipment is essential at the end of the session before storage to prevent growth of organisms during this time. The method described is rapid, taking no longer than four minutes to perform by an endoscopy assistant, and thus is easily accommodated into a busy endoscopy list.

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