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# Bactericidal, Virucidal, and Mycobactericidal Activities of Reused Alkaline Glutaraldehyde in an Endoscopy Unit

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**Baths with 2% alkaline glutaraldehyde are often reused for 14 days to decontaminate flexible fiberoptic endoscopes (FFEs) between patients, but the effect of such reuse on the disinfectant's activity has not been known. Many busy endoscopy units also disinfect FFEs with contact times shorter than those recommended by the disinfectant manufacturer. We therefore collected samples of the disinfectant over the 14-day reuse period from two manual and one automatic bath used for bronchoscopes and gastroscopes at a local hospital. Control samples were also collected from a manual bath of 2% alkaline glutaraldehyde which did not receive any endoscopes. The germicidal activities of the samples were assessed in a carrier test against a mixture of hepatitis A virus, poliovirus 1 (Sabin), and *Pseudomonas aeruginosa*; the mixture also contained either *Mycobacterium bovis* or *Mycobacterium gordonae*. Bovine serum (5%) was the organic load. The criterion of efficacy was a minimum of a 3-$\log_{10}$-unit reduction in the infectivity titers of the organisms tested. The initial disinfectant concentration in all the baths was nearly 2.25%; it became about 1.8% in the control bath and fell to approximately 1% in the three test baths after 14 days. No protein was detected in the control bath, while its concentration rose gradually in the test baths to a maximum of 1,267 μg/ml after 14 days. With a contact time of 10 min at 20 ± 2°C, all the samples from the control bath were effective against all the test organisms and all the samples from all the test baths were also effective against *P. aeruginosa*. With a contact time of 10 or 20 min at 20 ± 2°C, the virucidal and mycobactericidal activities of the samples from the test baths became variable after the seventh day. However, all the samples from the test baths showed broad-spectrum germicidal activity when the contact time was increased to 45 min and the temperature was raised to 25°C. These findings emphasize the care needed in the disinfection of FFEs, especially in view of the increasing threat of AIDS and the resurgence of tuberculosis.**

Heat-sensitive medical devices, such as flexible fiberoptic endoscopes (FFEs), require decontamination by chemicals between patients. With the continuing rise in the number of endoscopy procedures (4) and the increasing threat of infections caused by the human immunodeficiency virus and mycobacteria (2, 22), chemical disinfection of FFEs must be monitored carefully. A multistate investigation in the United States (25) often found disinfection procedures for endoscopes to be suboptimal, and nearly 24% of the samples obtained from internal channels of FFEs had bacterial contamination of at least $10^4$ CFU. Recent surveys of FFE-associated infections (40, 41, 43) have concluded that FFEs are a potential means of infection spread.

During reuse, the germicidal activity of a chemical disinfectant can be affected by a number of factors. Dilution of the chemical to levels below those that are recommended for its use may occur. The concentration of alkaline glutaraldehyde (2 to 2.5%), a disinfectant most commonly used for the disinfection of semicritical instruments, was shown to fall to <1% after 20 endoscopes were processed in a manual bath (22). Higher dilutions of the disinfectant occur in automatic disinfectors, and a fall from 2.4 to 0.5% after 26 to 30 cycles has been recorded (26). A Canadian study (45) reported similar findings. This evidence raises concern of alkaline glutaraldehyde efficacy over its recommended reuse period.

Apart from the dilution, contact time, pH level, organic load, aging, and the temperature at which alkaline glutaraldehyde is used are important for its germicidal activity (17). The number of instruments subjected to a chemical under reuse conditions also contributes to its loss of activity (22, 26, 45). In the present study, five different organisms were selected for use in monitoring the germicidal activity of alkaline glutaraldehyde during its reuse in an endoscopy unit. The disinfectant samples were also tested for their pHs, glutaraldehyde concentrations, and protein accumulation.

## MATERIALS AND METHODS

**Cells.** A seed culture of FRhK-4 cells was received from M. D. Sobsey, University of North Carolina, Chapel Hill. The methods for the cultivation and maintenance of these cells have been described previously (30, 31). Briefly, Eagle minimum essential medium (GIBCO, Grand Island, N.Y.) with 10% fetal bovine serum (FBS; GIBCO), 2 mM glutamine (GIBCO), 0.1 mM nonessential amino acids (GIBCO), 50 μg of gentamicin sulfate (Cidomycin; Roussel, Montreal, Quebec, Canada) per ml, 100 μg of kanamycin (GIBCO) per ml, 0.015 M HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid; GIBCO), and 0.113% sodium bicarbonate (BDH, Toronto, Ontario, Canada) was used for growing the cells. The cells were maintained in the same medium but containing only 2% FBS.

**Viruses.** The HM-175 strain of hepatitis A virus (HAV) was also received from M. D. Sobsey, and the Sabin strain of poliovirus type 1 (PV) was obtained from the Laboratory

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Center for Disease Control (LCDC), Ottawa, Ontario, Canada. The stocks of the viruses were prepared as described previously (30), except that the cells were maintained in a medium without antibiotics to avoid inhibition of the bacteria to be added to the mixture (see below). Virus plaque assays were carried out in FRhK-4 cell monolayers in 12-well plastic plates (Costar, Cambridge, Mass.) as described previously (30).

**Organic load.** The FBS that was used to culture FRhK-4 cells was obtained from one bottle, heat inactivated at 56°C for 30 min, cooled, and divided into 0.5-ml amounts and stored at −20°C.

***Pseudomonas aeruginosa.*** *P. aeruginosa* ATCC-27853, which was received from the LCDC, was used throughout the study. Stock cultures were grown 37°C for 48 h in Trypticase soy broth (Difco) in 500-ml flasks mounted on a shaker (Lab-Line Instruments; Melrose Park, Ill.). The cultures were washed three times in fresh broth before preservation. Day-to-day cultures were grown by inoculating a single drop from a stock culture into 12 ml of Trypticase soy broth in a glass vial and were incubated at 37°C for 48 h, with the screw cap loose. The cultures were vortexed (Vortex Genie 2; Fisher Scientific) once after 24 h of incubation. *P. aeruginosa* colonies were enumerated by spreading 0.1-ml quantities of each test sample onto the surfaces of three plastic petri dishes (100 mm) of Bacto *Pseudomonas* Agar F (Difco). Colony counts were recorded after an incubation period of 24 h at 37°C and again on the third day of incubation.

**Mycobacteria.** *Mycobacterium bovis* BCG (ATCC-35743) was obtained from the LCDC. It is widely used as a surrogate to test the tuberculocidal activities of liquid chemical disinfectants (7). *Mycobacterium gordonae* NRCT-1101 was also received from the LCDC. This organism is reported to be more resistant than *Mycobacterium tuberculosis* (15), suggesting that its susceptibility to a germicide can be extrapolated to tubercle bacteria. Moreover, it is a relatively safe organism to work with because it is the least pathogenic of the Runyon group II (24) and a saprophyte commonly found in the soil (44, 46).

Stock cultures of both *M. bovis* and *M. gordonae* were grown as shake cultures in the same way as described above for *P. aeruginosa*, except that Bacto Middlebrook 7H9 broth (Difco) containing 0.5% Tween 80 (Baker Chemical Co., Phillipsburg, N.J.) supplemented with Bacto Middlebrook OADC enrichment (Difco) was used and growth was allowed to occur for 21 days at 37°C, with vortex mixing every 2 days. Such cultures were washed separately three times in fresh broth before preservation as stocks. The day-to-day cultures were grown in 12 ml of Bacto Middlebrook 7H9 broth containing 0.5% Tween 80 and were supplemented as described above in screw-cap glass vials held at 37°C. Bacterial enumeration was done by spreading out 0.1-ml quantities of a culture onto Bacto Middlebrook 7H11 agar containing 0.5% glycerol (BDH) and incubation at 37°C. Colonies were counted on day 14 for *M. gordonae* and on day 21 for *M. bovis*. A second and final count was made on day 30. Gentamicin (Hoechst-Roussel, Montreal, Quebec, Canada) at a final concentration of 15 μg/ml or 150 μl of piperacillin (Cyanamid Canada Inc., Montreal, Quebec, Canada) per ml was added to Bacto Middlebrook 7H11 agar when the medium was used for the detection of the mycobacteria in the mixture while suppressing the growth of *P. aeruginosa*.

**Preservation of the bacterial stocks.** The washed bacterial cells were suspended in fresh broth containing 7% glucose (BDH) and 15% heat-inactivated FBS in 2-ml plastic vials that were brought to −20°C overnight before storing them at −80°C.

**Glass cups as carriers.** Glass cups (1 cm in diameter and height) were cut out of the bottom of Pyrex serum dilution tubes measuring about 1 cm in diameter and 7.5 cm in height. Before reuse, the glass cups were soaked in 10% nitric acid overnight at room temperature. They were then thoroughly rinsed under running tap water and were soaked in 7× cleaning solution (Fisher) for 24 h. The detergent was rinsed thoroughly under running deionized distilled water. The glass cups were allowed to dry at room temperature and were then baked at 160°C for 2 h in a glass beaker covered with aluminum foil.

**Glutaraldehyde.** Cidex, a product containing about 2% glutaraldehyde, was purchased by the Ottawa General Hospital (Ottawa, Ontario, Canada) from Johnson & Johnson Medical, Inc. (Peterborough, Ontario, Canada) for use in the Endoscopy Unit. The activator provided with the product was added to it just prior to filling the disinfectant baths. The directions for use on the product label state that items to be disinfected are to be immersed in it completely for a minimum of 10 min at 20°C to destroy vegetative organisms including *P. aeruginosa*, pathogenic fungi, and enveloped as well as nonenveloped viruses; for 100% killing of *M. tuberculosis*, the contact time recommended on the label is 45 min at 25°C.

**Disinfectant baths.** The disinfection of endoscopes at the hospital includes the following procedure: a prewash in warm tap water containing an enzymatic detergent (Metrizyme; Metrex Research Corp., Vancouver, British Columbia, Canada), a rinse in fresh warm tap water, and exposure to the disinfectant without any drying. Two manual baths and one automatic machine (EW-20; Olympus, Tokyo, Japan) for the disinfection of gastroscopes and bronchoscopes at the Ottawa General Hospital were selected for the present study. The manual baths were basins provided with Cidex (Johnson & Johnson). Each bath was carefully washed and dried before it was filled with 4 liters of freshly activated 2% alkaline glutaraldehyde. The automatic bath was washed and disinfected before adding 2 liters of fresh detergent into the detergent compartment and 15 liters of the disinfectant solution. The disinfectant was reused for 14 days in all the baths. For a control, a manual bath was set up with 4 liters of freshly prepared alkaline glutaraldehyde solution and placed in the same room as the test baths. The main steps in the processing of the disinfectant samples in our laboratory are summarized in Fig. 1.

**Collection of disinfectant samples.** Approximately 15 ml of the disinfectant was collected daily from each bath except on days 1 and 2 (Saturday and Sunday, respectively) because the 14-day cycle at the hospital starts on a Friday. Samples from the automatic bath were taken promptly within 3 min of the start of the 20-min disinfection phase. The solution in the manual baths was first thoroughly mixed before withdrawing the samples. The samples were transported to our laboratory (next door to the hospital) for immediate processing.

**Microbial elution.** A full-strength Bacto Middlebrook 7H9 broth containing 2% glycine was used as the eluent. A combination of this broth and glycine (7H9G) was found to be suitable for neutralizing the disinfectant. Initial experiments were carried out by incubating a mixture of *M. gordonae*, *P. aeruginosa*, HAV, and PV in 5 ml of 7H9G for 4.5 h in a laminar flow hood. No significant loss in the viability of the test organisms was detected when compared

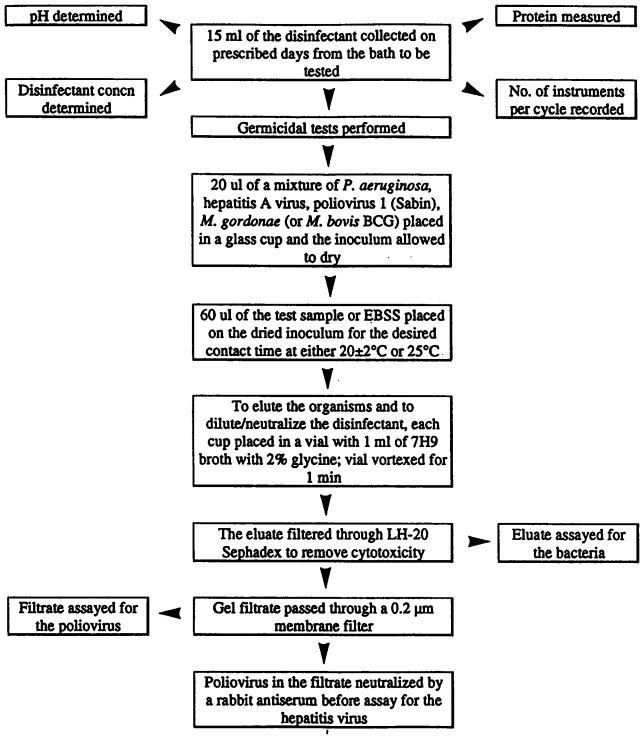


FIG. 1. Flow chart of the main steps in the processing of alkaline glutaraldehyde samples collected at the Endoscopy Unit. EBSS, Earle balanced salt solution.

with that of a control comprising 7H9 broth without glycine. A parallel test with *M. bovis* gave similar results.

**Preparation of mixed inocula.** Twelve milliliters of a 21-day-old culture of *M. gordonae* was mixed with an equal amount of a 28-h-old culture of *P. aeruginosa*. The mixture was then centrifuged at 3,000 × *g* for 60 min. The supernatant was discarded in a jar of 2% sodium hypochlorite. The bacterial pellet was resuspended in approximately 1.5 ml of a mixture of HAV and PV. Each milliliter of the bacteria-virus mixture contained about $10^6$ PFU of HAV, $10^7$ PFU of PV, $10^{8.5}$ CFU of *P. aeruginosa*, and $10^{6.5}$ CFU of *M. gordonae* or $10^7$ CFU of *M. bovis*. Since the virus pools contained 2% FBS, an additional amount of FBS was added to the mixture to bring the final concentration of serum to 5%.

**Carrier test for germicidal activity.** The bacteria-virus mixture was vortexed, and 20 μl was carefully placed at the bottom of each glass cup held in a well of a 96-well microtiter plate (Costar). The inocula were allowed to dry at ambient temperature and relative humidity for 60 min in a laminar flow hood. Sixty microliters of the disinfectant (controls received 60 μl of Earle balanced salt solution instead) was placed on the dried inoculum by using a positive displacement pipet (Gilson Medical Instruments, Villiers-le-Bel, France), and the mixture was allowed to act for 10 or 20 min at ambient temperature (20 ± 2°C) or for 45 min at 25°C in an incubator. At the end of the desired contact time, each glass cup was dropped into a glass vial containing 1 ml of 7H9G. The vial was gently vortexed for 1 min before pipetting its contents up and down five times to ensure complete elution. Experiments with *M. bovis* alone and in a mixture containing PV, HAV, and *P. aeruginosa* were done in parallel and in the same way as described above for the mixed culture containing *M. gordonae* instead. This procedure is summarized in Fig. 1.

**Removal of residual disinfectant.** The removal of the disinfectant from the eluates was done by the procedure of Blackwell and Chen (8). The eluates were loaded onto preswollen (equilibrated) Sephadex LH-20 gels (Pharmacia, Uppsala, Sweden) in plastic cones (Amicon Corp., Lexington, Mass.) placed in 50-ml plastic centrifuge tubes. The tubes were centrifuged at 2,000 × *g* for 5 min. When the microorganisms were suspended in the eluent (without disinfectant) and passed through the gel, the recovery rates were 78, 76, 92, 95, and 77% for *M. gordonae*, *P. aeruginosa*, HAV, PV, and *M. bovis*, respectively.

**Enumeration of surviving microorganisms.** A 0.1-ml amount of the gel filtrate was spread onto each bacteriological medium for the detection and enumeration of the bacteria in the mixture.

The remaining gel filtrate was passed through a 0.2-μm-pore-size membrane filter (Nalgene Co., Rochester, N.Y.) to remove the bacteria before plaque assay for the viruses. For the quantitation of PV, the membrane filtrate did not require any further manipulations. However, for HAV plaque assay, the PV in the mixture was first neutralized by incubating the mixture for 1 h at 37°C with an equal volume of diluted rabbit hyperimmune anti-PV serum provided by P. Payment of the Institut Armand-Frappier, Montreal, Quebec, Canada. The undiluted serum had a 50% PFU reduction titer of greater than 1:30,000, and the final dilution of serum used was pretested to neutralize nearly $10^6$ PFU of PV.

**Estimation of disinfectant pH.** The pH of each disinfectant sample was determined with the help of a Fisher Scientific (Ottawa, Canada) pH meter (model 220).

**Estimation of protein accumulation.** Estimations of protein in the disinfectant samples were made by using a modification (33) of the method of Lowry et al. (27).

**Estimation of glutaraldehyde concentration.** We chose to use the following method after extensive consultations with the manufacturers of glutaraldehyde-based products. The protocol was kindly supplied to us by Surgikos Canada, Inc. (Peterborough, Ontario, Canada), and it is based on the reaction of alkaline glutaraldehyde with hydroxylamine to produce hydrochloric acid as one of the by-products, which is then titrated against 0.1 N NaOH.

**Cumulative loading of instruments.** The Endoscopy Unit at the hospital was requested to maintain an accurate record of the number of instruments processed daily through those reuse cycles of the baths that acted as the sources of the disinfectant samples for the present study. The cumulative number of instruments for the cycles for each one of the three types of baths is presented in Fig. 2.

**Rating of germicidal activity.** A given sample of the disinfectant was considered effective if it could reduce the infectivity titer of the test organism(s) by at least 3 $\log_{10}$ units under our test conditions. This arbitrary criterion for germicidal efficacy of the product is accepted by many regulatory agencies and is included as a part of the standard protocols of the Canadian General Standards Board (11). Disinfectant samples were collected from seven complete cycles of the manual bronchoscope and gastroscope baths and four cycles of the automatic bath. The control samples came from one manual bath.

## RESULTS

**pH levels.** The mean pH values of the disinfectant control and the samples from the three types of baths are presented

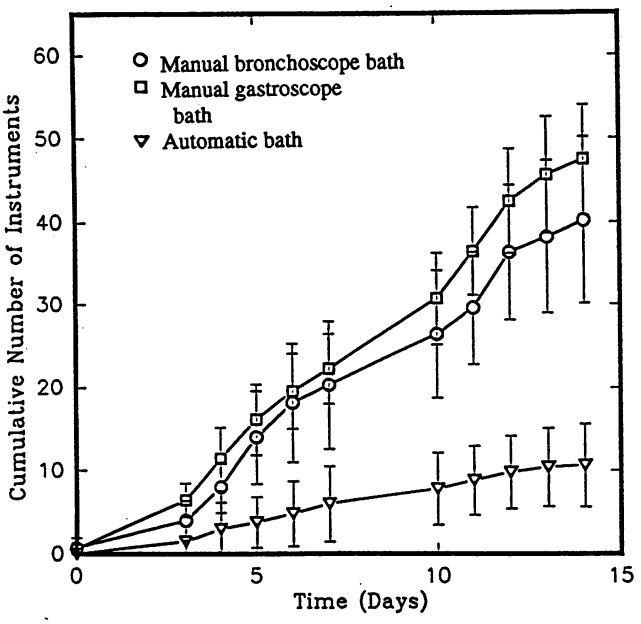


FIG. 2. Cumulative numbers of instruments in endoscope disinfection baths during a 14-day reuse period.

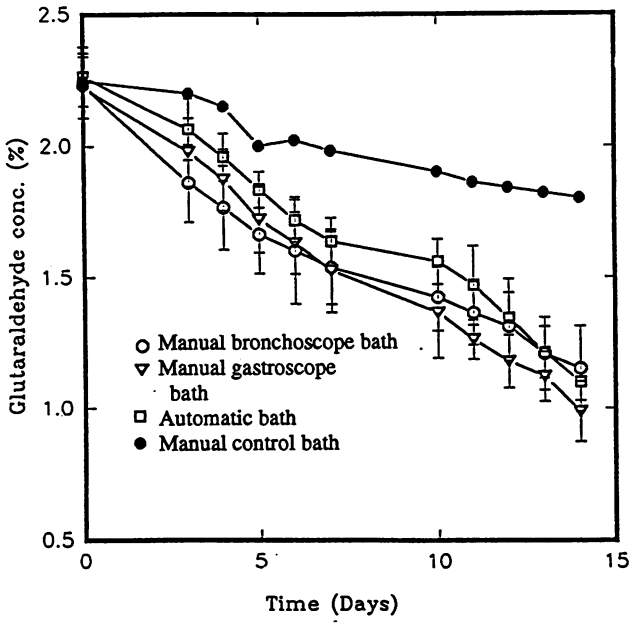


FIG. 4. Alkaline glutaraldehyde concentration (in percent) in endoscope disinfection baths during a 14-day reuse period. Vertical error bars represent standard deviations from the mean.

in Fig. 3. The pH of the control sample was 8.5 at the outset and showed no significant change over the test period. At the beginning of the cycle, the pH of all the samples from the three baths was approximately 8.4. The samples from the automatic bath showed the most pronounced drop in pH early in the reuse cycle, and the reduction continued over the 14-day period; the pH on day 14 was 7.7 ± 0.2. On the other hand, the pHs of the samples from the bronchoscope bath remained fairly constant, with the pH on day 14 being 8.3 ± 0.2. The pHs of the samples from the gastroscope bath showed a certain degree of fluctuation over the first 10 days

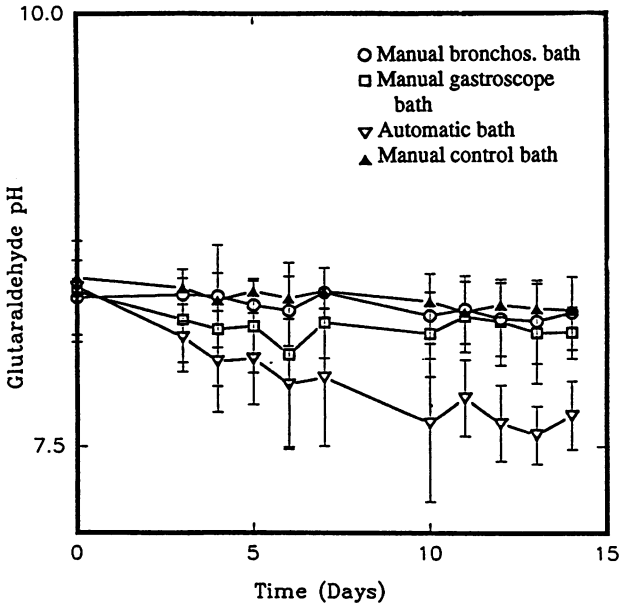


FIG. 3. Alkaline glutaraldehyde pH levels in endoscope disinfection baths during a 14-day reuse period.

of reuse, but remained essentially unaltered for the last 4 days, with the final pH being 8.2 ± 0.2.

**Glutaraldehyde concentration.** As can be seen from Fig. 4, the glutaraldehyde concentration in the control sample went from 2.25% at the beginning to a low of 1.8% on day 14. The mean initial concentrations of the disinfectants in the samples from the three test baths were between 2.23 ± 0.12 and 2.27 ± 0.11%, with a range of 2.06 to 2.37%. Irrespective of the type of the test bath, there was a gradual decline in glutaraldehyde concentration over the period of reuse, and at the end of day 14, the levels recorded were 1.15 ± 0.16, 0.99 ± 0.11, and 1.1 ± 0.07% for the manual bronchoscope, the manual gastroscope, and automatic baths, respectively.

**Protein concentration.** Whereas no protein could be detected in any of the samples from the control bath, the levels of protein gradually increased over the reuse period in the samples from the three test baths (Fig. 5). This increase was somewhat slow in the first 5 days, with higher rates of accumulation during the remaining period of reuse. At the end of day 14, the values for protein concentration were 1,074.2 ± 110.6, 1,067.6 ± 139.1, and 663.4 ± 60.0 μg/ml for the bronchoscope, gastroscope, and automatic baths, respectively.

**Germicidal activity.** The samples from the control bath remained effective against all the test organisms after 14 days, even at the shortest (10 min) contact time tested. Those from the three test baths were able to reduce the infectivity titer of the five types of microorganisms to undetectable levels from days 0 to 6 of reuse. However, there were considerable variations in the disinfectant's broad-spectrum germicidal activity after this period of reuse. It is also worth noting here that once samples in a particular cycle began to fail against a test organism, the activity never showed an improvement over the rest of the cycle. There were no significant differences in reused alkaline glutaraldehyde activity against the two mycobacteria or HAV at contact times of 10 and 20 min. All disinfectant samples from

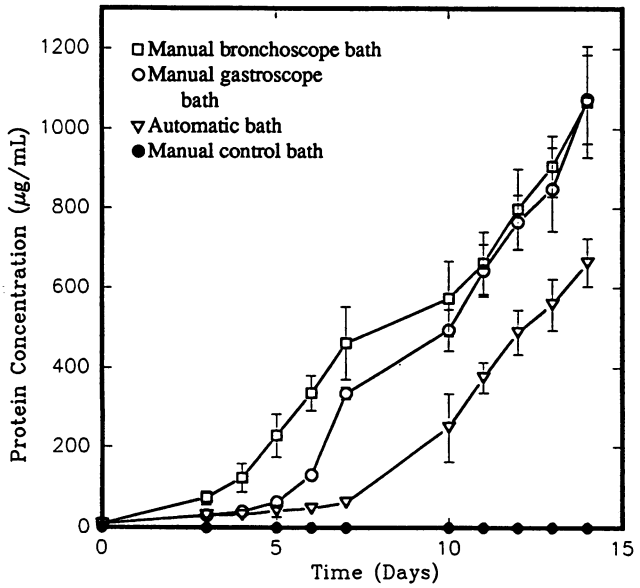


FIG. 5. Protein accumulation in disinfection baths during a 14-day reuse period. Vertical error bars represent standard deviations from the mean.

TABLE 2. Days of reuse at which samples of alkaline glutaraldehyde showed the first failure

| Test organism | Contact time (min)[a] | Days of reuse | | | |
|---|---|---|---|---|---|
| | | Manual bath | | Automatic bath | Control bath |
| | | Broncho-scopes | Gastro-scopes | | |
| *P. aeruginosa* | 10 | >14[b] | >14 | >14 | >14 |
| PV | 10 | 12 | 12 | 13 | >14 |
| HAV | 10 | 11 | 11 | 10 | >14 |
| | 20 | 11 | 11 | ND[c] | ND |
| | 45 | >14 | >14 | ND | ND |
| *M. bovis* | 10 | 14 | 12 | 12 | >14 |
| | 20 | 14 | 13 | ND | ND |
| | 45 | >14 | >14 | ND | ND |
| *M. gordonae* | 10 | 11 | 7 | 10 | >14 |
| | 20 | 11 | 7 | ND | ND |
| | 45 | >14 | >14 | ND | ND |

[a] For contact times of 10 and 20 min, the temperature was 20 ± 2°C, and for a contact time of 45 min, the temperature was 25°C.
[b] >14, effective for reuse life of the disinfectant bath.
[c] ND, not done.

all the baths remained effective against *P. aeruginosa* over the entire reuse period. The following highlights the differences recorded in the germicidal activities of the samples from the three test baths.

(i) **Manual bronchoscope disinfection bath.** As can be seen from Table 1, samples from the manual bronchoscope disinfection bath, when tested by using a contact of 10 min, remained effective against *M. bovis* until the end of day 13, with 1 sample of 7 failing on day 14. Failure (one of seven samples) of disinfectant activity against *M. gordonae* first appeared at the end of day 11 and became progressively worse over the remaining period of reuse; at the end of day 14, four of the seven samples did not meet the efficacy requirements. At a contact time of 10 min, the samples from the manual bronchoscope disinfection bath remained effective against HAV and PV until the end of days 10 and 11, respectively. Extending the contact time to 20 min did not improve the picture with regards to *M. bovis*, *M. gordonae*, and HAV (Table 2). However, when the contact time was increased to 45 min and the temperature was raised to 25°C, all samples from this bath remained effective against HAV and the two mycobacteria (Table 2) until the end of the reuse period.

TABLE 1. Number of disinfectant samples on a given day of the reuse cycle unable to meet the germicidal efficacy criterion against the organism(s) in the mixture[a]

| Bath type and test organism | Number of disinfectant samples on the following day of disinfectant sample collection | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6 | 7 | 10 | 11 | 12 | 13 | 14 |
| Manual bath for broncho-scopes (seven cycles) | | | | | | | |
| PV | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| HAV | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| *M. bovis* | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *M. gordonae* | 0 | 0 | 0 | 1 | 2 | 3 | 4 |
| Manual bath for gastroscopes (seven cycles) | | | | | | | |
| PV | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| HAV | 0 | 0 | 0 | 1 | 1 | 2 | 3 |
| *M. bovis* | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *M. gordonae* | 0 | 2 | 2 | 4 | 4 | 4 | 4 |
| Automatic bath for broncho-scopes and gastroscopes (four cycles) | | | | | | | |
| PV | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| HAV | 0 | 0 | 1 | 2 | 3 | 3 | 3 |
| *M. bovis* | 0 | 0 | 0 | 0 | 1 | 2 | 2 |
| *M. gordonae* | 0 | 0 | 2 | 2 | 3 | 3 | 3 |

[a] The contact time was 10 min at 20 ± 2°C.

(ii) **Manual gastroscope disinfection bath.** At the contact time of 10 min, the samples from the manual gastroscope disinfection bath began to fail (two of seven samples) against *M. gordonae* starting on day 7, and the failure became progressively worse (Table 1); with *M. bovis*, two of the seven samples could not meet our efficacy criterion at the end of day 12. The first failures against HAV and PV were recorded at the end of days 11 and 12, respectively, when the contact time was 10 min (Table 1). Extending the contact time to 20 min did not improve the performance of the samples against *M. gordonae* and HAV, whereas the first failure against *M. bovis* was recorded (Table 2) 1 day later than that seen with a contact time of 10 min. With a contact time of 45 min at 25°C, all the samples from the manual gastroscope disinfection bath could meet our efficacy criterion (Table 2).

(iii) **Automatic bath.** Samples from only four 14-day cycles could be collected from the automatic bath. All of the samples from the automatic bath as well remained effective against *P. aeruginosa*. The first two failures against *M. gordonae* were noted at the end of day 10, when the contact time was 10 min (Table 1). Although the samples proved to be ineffective against *M. bovis* from the end of day 12, these

failures were marginal because the reductions in the infectivity titer were only slightly less than 3 $\log_{10}$ units. As can be seen from the results of the virucidal tests summarized in Table 1, the activity against PV remained undiminished until the end of day 12, whereas failures against HAV began to appear 3 days earlier.

## DISCUSSION

Although iodophors and alcohols are some times used to decontaminate semicritical medical instruments between patients (12, 19, 39), 2% alkaline glutaraldehyde is presently the most widely used disinfectant for this purpose (39). The activity of alkaline glutaraldehyde against microorganisms is well established (6, 29, 42). There is, however, growing concern over the possible negative influence of disinfectant dilution, pH changes, and protein accumulation in reuse baths on the germicidal activity of alkaline glutaraldehyde (6, 13, 18). Furthermore, there are no officially recognized test protocols for premarket evaluation of disinfectant products meant for reuse.

During the 14-day reuse period in the present study, the concentration of the disinfectant was noted to fall steadily, and in all the test baths its level reached close to 1%. The Sterilog Monitor, which shows a change of color on chemically impregnated pads if the glutaraldehyde solution is still active, failed to detect this drop in the disinfectant concentration. Power and Russell (34) also found the Sterilog Monitor to be unreliable as an indicator of glutaraldehyde concentration.

At the end of the 14-day reuse period, the mean disinfectant level in the automatic bath was 1.1 ± 0.07%. Others have shown the levels of the chemical to fall below 1% (45) to as low as 0.27% (26) on day 4 of reuse. The discrepancy between our results and those of the other groups may be due to the number of instruments that were washed and disinfected in the automatic bath; in our study, 4 to 16 instruments were put through the bath in a 14-day cycle, whereas the number of instruments recorded for the same period was 60 and 52 to 60 by Whyman et al. (45) and Leong et al. (26), respectively. The mean alkaline glutaraldehyde concentration of just over 1.0% after 4 to 16 machine cycles observed in the present study emphasizes the fact that automatic baths severely dilute the product, and the level of the disinfectant in these machines should be closely monitored with reliable laboratory tests. Most hospitals that use alkaline glutaraldehyde for disinfection of FFEs do not monitor its concentration during its period of reuse (25).

Whereas titremetric (3) and spectrophotometric (21, 34) methods are available for the estimation of fresh alkaline glutaraldehyde (18), they all suffer from certain drawbacks and have not been evaluated under reuse conditions. However, titremetric procedures are easy to perform, and one involving double indicators to improve accuracy could be developed to estimate the glutaraldehyde concentration during reuse.

In the manual baths, the relationship between the number of instruments disinfected and the dilution of the disinfectant was not as dramatic. After disinfection of 30 to 54 instruments and on day 14, the level of glutaraldehyde in the bronchoscope bath was 1.15 ± 0.16%, whereas that in the gastroscope bath was 0.99 ± 0.11% after disinfection of 41 to 59 instruments. Bageant et al. (5) found as much as 250 to 300 ml of rinse water trapped in automatic baths, and this may contribute to disinfectant dilution. It has been suggested that the rubber and tubings in the automatic bath may also absorb the disinfectant (34). A drop in disinfectant level occurs, perhaps because of evaporation as well as polymerization, with the age of the solution, and this is clearly indicated by the drop in the concentration of disinfectant in the standing control (Fig. 4).

Alkaline glutaraldehyde shows better germicidal activity at about pH 8.0 (34). The drop in the pH of the disinfectant solution was most prominent in the automatic bath (Fig. 3), and again, this may be related to the higher dilution of the disinfectant with the rinse water.

There was a substantial increase with reuse in the levels of protein in all three test baths (Fig. 5). Higher levels of protein accumulation (0.02 to 0.1%) have been reported in the only other study to estimate protein accumulation during disinfectant reuse (35). Alkaline glutaraldehyde is considered to be more resistant to interference by organic matter than most other disinfectants (17, 36), even though its germicidal action may be due to its reaction with the protein components of microorganisms (18). As can be seen from Fig. 3 and 4, there was an inverse relationship between protein accumulation and glutaraldehyde concentration.

Samples from the control bath remained effective against all test organisms over the 14-day period of disinfectant aging. On the basis of the data presented in Table 1, a preliminary rating, in decreasing order of resistance to reused alkaline glutaraldehyde, of the four organisms in the mixture would be *M. gordonae*, HAV, PV, and *P. aeruginosa*. *M. bovis* proved to be less resistant to reused alkaline glutaraldehyde than *M. gordonae* when tested in a mixture. Moreover, similar results for *M. bovis* tested in the mixture or alone (data not shown) suggest that no undue resistance is conferred upon the bacteria by the presence of other organisms in the inoculum. There were definite advantages associated with longer contact times at higher temperatures (45 min at 25°C) with respect to all the organisms tested. The label claim for the disinfectant indicates that this temperature and contact time are tuberculocidal. We did not test *M. tuberculosis* under these conditions, but *M. gordonae* is reported to be more resistant than *M. tuberculosis* to fresh glutaraldehyde (15). Hence, the manufacturer's tuberculocidal efficacy label claims at 25°C for contact times of 45 min were reproducible even under reuse conditions, even though many endoscopy units find it difficult to use this extended contact time and the higher temperature on a routine basis. In fact, the contact time used to disinfect endoscopes is variable and can be as short as 4 min (4, 14). A contact time of 20 min at room temperature is the standard practice for disinfection of endoscopes between patients in our hospital.

Protein accumulation and the number of instruments disinfected in the manual baths increased significantly by day 7, while the level of the disinfectant and pH also fell markedly (Fig. 3 and 4). At the same time, its germicidal activity commenced to show variable failure rates against the tested organisms (Table 1). Hence, there is an apparent correlation between these factors and germicidal activity. We are not aware of any other published studies in which such a relationship has been demonstrated. Robinson et al. (36) examined the germicidal activities of glutaraldehyde-based products in dental clinics and found that both acid and alkaline solutions of glutaraldehyde retained their germicidal activities against *Salmonella choleraesuis*, *Staphylococcus aureus*, and *P. aeruginosa* over 24 days of reuse; they used a suspension test to assess germicidal activity, and the three species tested were all relatively susceptible vegetative bacteria. Our study used a more realistic carrier test and a

mixture of microorganisms with various degrees of resistance to chemical disinfectants.

A number of microorganisms have been used to assess the efficacy of alkaline glutaraldehyde (5, 13, 15, 23). PV has been recommended as the prototype for determining the virucidal activity of chemical disinfectants (11). However, HAV has been shown to be particularly resistant to a wide variety of disinfectants (29). Although this virus was susceptible to freshly activated alkaline glutaraldehyde in a carrier test (29), it was not known whether this would remain the case upon reuse of the product. Moreover, there is opportunity for HAV contamination of FFEs from an asymptomatic patient(s) undergoing an endoscopic procedure (37). We therefore sought to compare PV resistance to alkaline glutaraldehyde under reuse with that of HAV.

*P. aeruginosa* can cause severe and often fatal disease in immunocompromised patients. Those suffering from cystic fibrosis, cancer, diabetes, or heart diseases are particularly at risk. For instance, in 90% of patients with cystic fibrosis, *P. aeruginosa* is shown to be the primary pathogen (28) and is the most common nosocomial pathogen in patients hospitalized for more than 1 week (9). Moreover, among opportunistic infections with gram-negative bacilli, those caused by *P. aeruginosa* are associated with particularly high mortality (28). It has also been isolated from channels of endoscopes disinfected in automatic baths (1, 2).

Cases of tuberculosis are on the rise again in industrialized countries, and infections caused by atypical mycobacteria are also being reported with increasing frequency, particularly in association with AIDS and immunosuppression (46). This has further enhanced the urgency of instituting effective measures for the prevention and control of such infections.

The chemicals to be used for the disinfection of semicritical instruments must be shown to be effective against mycobacteria (16, 38). The test protocols for the assessment of such activity recommend the use of *Mycobacterium smegmatis* for the initial tests and then confirmatory tests with *M. bovis* (7). There is, however, considerable disagreement over the suitability of these two organisms as surrogates for *M. tuberculosis*. As a result, a number of other mycobacteria have been tested as potential substitutes. The notable species in this regard are *Mycobacterium terrae* (20), *Mycobacterium avium* and *Mycobacterium fortuitum* (15), and *Mycobacterium chelonae*. These species were considered unsuitable for use in the present study because of various factors, such as their potential pathogenicity to humans (32, 46) and variability in resistance to alkaline glutaraldehyde (20). Instead, we chose to include *M. gordonae* as a representative of the mycobacteria because it is considered as the least pathogenic of the Runyon group II mycobacteria (24).

The studies by Isenberg et al. (23) and Bageant et al. (5) attempted to assess the effect of instrument and organic load on alkaline glutaraldehyde under simulated clinical use. The germicidal activity of alkaline glutaraldehyde against *M. bovis*, *M. smegmatis*, *S. aureus*, *P. aeruginosa*, and *Candida albicans* remained over the entire reuse period of 14 days; the instruments used by them were not used on patients prior to soaking in the disinfectant bath. Our study, on the other hand, determined the germicidal activities of disinfectant samples collected from baths in an actual clinical setting. What our test actually measured was the potential for disinfectant efficacy on the next instrument to be processed in the reuse bath.

The findings reported here suggest that 2% alkaline glutaraldehyde may become ineffective against nonenveloped viruses and mycobacteria in much less than 14 days in reuse baths meant for the disinfection of semicritical medical instruments such as fiberoptic endoscopes if the instruments are exposed to it for 10 to 20 min at room temperature. Inadequate decontamination of such instruments between patients increases the risk of disease transmission. Particular attention must be paid to improving the basic design and functioning of the automatic bath because, in its present form, it appears to be incompatible with the recommended use of the disinfectant. During the course of the present study, the bath tended to break down frequently and the personnel at our hospital often avoided its use. In certain other countries (10), FFE disinfection baths with 2% alkaline glutaraldehyde are reused for no longer than 7 days.

It has been suggested that infections associated with endoscopic procedures (1, 2, 40) may have been due to failure of the disinfection procedure. The risk of spread of infections through improperly disinfected endoscopes is on the rise because of the continuing increase in the number of such procedures and the number of cases of infections caused by mycobacteria, HAV, and human immunodeficiency virus. In view of this, better protocols are urgently needed to assess the broad-spectrum germicidal activities of disinfectants to be reused in the decontamination of semicritical instruments.

*M. gordonae* and HAV were the most resistant organisms tested in the present study. Their susceptibilities to alkaline glutaraldehyde reflected the susceptibilities of the other organisms. A mixed culture of only *M. gordonae* and HAV may suffice in the reuse test for alkaline glutaraldehyde in the glass carrier test. In the present study, the results obtained with these two organisms were predictive of broad-spectrum activity against bacteria and viruses, and such a test can be further evaluated on an interlaboratory basis to constitute the basis for a standard germicidal test procedure for products meant for reuse.

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## REFERENCES

1. **Allen, J. I., M. O. Allen, M. M. Olson, D. N. Gerding, C. J. Shanholtzer, P. B. Meier, J. A. Vennes, and S. E. Silvis.** 1987. *Pseudomonas* infection of the biliary system resulting from use of a contaminated endoscope. Gastroenterology **92**:759–763.
2. **Alvarado, C. J., S. M. Stolz, and D. G. Maki.** 1991. Nosocomial infection and pseudoinfection from contaminated endoscopes and bronchoscopes—Wisconsin and Missouri. Morbid. Mortal. Weekly Rep. **40**:675–677.
3. **Anderson, P. J.** 1967. Purification and quantitation of glutaraldehyde and its effect on several enzyme activities in skeletal muscle. J. Histochem. Cytochem. **15**:652–661.
4. **Axon, A. T. R.** 1991. Disinfection of endoscopic equipment. Bailiere's Clin. Gastroenterol. **5**:61–77.
5. **Bageant, R. A., F. J. Marsik, V. A. Kellogg, D. L. Hyler, and D. H. M. Gröschel.** 1981. In-use testing of four glutaraldehyde disinfectants in the cidematic washer. Respir. Care **26**:1255–1261.
6. **Bailly, J.-L., M. Chambon, H. Peigue-Lafeuille, H. Laveran, C. De Champs, and D. Beytout.** 1991. Activity of glutaraldehyde at low concentrations (<2%) against poliovirus and its relevance to gastrointestinal endoscope disinfection procedures. Appl. Environ. Microbiol. **57**:1156–1160.
7. **Beloian, A.** 1990. Disinfectants, p. 133–146. *In* Official methods of analysis of Association of Official Analytical Chemists.

Association of Official Analytical Chemists, Washington, D.C.
8. **Blackwell, O. H., and J. H. S. Chen.** 1970. Effects of various germicide chemicals on H.Ep cell culture and herpes simplex virus. J. Assoc. Off. Anal. Chem. **53**:1229–1236.
9. **Bodey, G. P., R. Bolivar, V. Fainstein, and L. Jadeja.** 1983. Infections caused by *Pseudomonas aeruginosa*. Rev. Infect. Dis. **5**:279–313.
10. **Bradley, C. (Dudley Road Hospital, Birmingham, England).** 1993. Personal communication.
11. **Canadian General Standards Board.** 1991. Assessment of efficacy of antimicrobial agents for use on environmental surfaces and medical devices. CAN/C.G.S.B.-2.161-M91. Canadian General Standards Board, Ottawa, Ontario, Canada.
12. **Carr-Locke, D. L., and P. Clayton.** 1978. Disinfection of upper gastrointestinal fibreoptic endoscopy equipment: an evaluation of a cetrimide chlorhexidine solution and glutaraldehyde. Gut **19**:916–922.
13. **Cole, E. C., W. A. Rutala, L. Nessen, N. S. Wannamaker, and D. J. Weber.** 1990. Effect of methodology, dilution, and exposure time on the tuberculocidal activity of glutaraldehyde-based disinfectants. Appl. Environ. Microbiol. **56**:1813–1817.
14. **Collignon, P. J., and E. Graham.** 1991. Cleaning and disinfection of endoscopes: have there been recent improvements? Med. J. Aust. **154**:391–394.
15. **Collins, F. M.** 1986. Bactericidal activity of alkaline glutaraldehyde solution against a number of atypical mycobacterial species. J. Appl. Bacteriol. **61**:247–251.
16. **Favero, M. S., and W. W. Bond.** 1991. Chemical disinfection of medical and surgical materials, p. 617–641. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation. Lea & Febiger, Philadelphia.
17. **Gelinas, P., and J. Goulet.** 1983. Neutralization of the activity of eight disinfectants by organic matter. J. Appl. Bacteriol. **54**:243–247.
18. **Gorman, S. P., E. M. Scott, and A. D. Russell.** 1980. Antimicrobial activity, uses and mechanism of action of glutaraldehyde. J. Appl. Bacteriol. **48**:161–190.
19. **Gorse, G. J., and R. L. Messner.** 1991. Infection control practices in gastrointestinal endoscopy in the United States: a national survey. Infect. Control Hosp. Epidemiol. **12**:289–296.
20. **Gundermann, K. O.** 1987. Sensitivity of different strains of mycobacteria to disinfectants of different compositions. Artzl. Lab. **33**:327–330.
21. **Hadju, J., and P. Friedrich.** 1975. Reaction of glutaraldehyde with $NH_2$ compounds. A spectrophotometric method for the determination of glutaraldehyde concentration. Anal. Biochem. **65**:273–280.
22. **Hanson, P. J. V., J. R. Clarke, G. Nicholson, B. Gazzard, H. Gaya, D. Gor, M. V. Chadwick, N. Shah, D. J. Jeffries, and J. V. Collins.** 1989. Contamination of endoscopes used in AIDS patients. Lancet **ii**:86–88.
23. **Isenberg, H. D., E. R. Giugliano, K. France, and P. Alperstein.** 1988. Evaluation of three disinfectants after in-use stress. J. Hosp. Infect. **11**:278–285.
24. **Jarikre, L. N.** 1991. Case report: disseminated Mycobacterium gordonae infection in a nonimmunocompromised host. Am. J. Med. Sci. **302**:382–384.
25. **Kaczmarek, R. G., R. M. Moore, Jr., J. McCrohan, D. A. Goldmann, C. Reynolds, C. Cacquelin, and E. Israel.** 1992. Multi-state investigation of the actual disinfection/sterilization of endoscopes in health care facilities. Am. J. Med. **92**:257–261.
26. **Leong, D., C. Dorsey, and M. Klapp.** 1991. Dilution of glutaraldehyde by automatic endoscope machine washers: the need for a quality control program. Am. J. Infect. Control **19**:86.
27. **Lowry, O. H., N. H. Rosenbrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
28. **Marshall, B. C., and K. C. Carroll.** 1991. Interaction between *Pseudomonas aeruginosa* and host defenses in cystic fibrosis. Semin. Respir. Infect. **6**:11–18.
29. **Mbithi, J. N., V. S. Springthorpe, and S. A. Sattar.** 1990. Chemical disinfection of hepatitis A virus on environmental surfaces. Appl. Environ. Microbiol. **56**:3601–3604.
30. **Mbithi, J. N., V. S. Springthorpe, and S. A. Sattar.** 1991. Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. Appl. Environ. Microbiol. **57**:1394–1399.
31. **Mbithi, J. N., V. S. Springthorpe, and S. A. Sattar.** 1992. Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. J. Clin. Microbiol. **30**:757–763.
32. **Peters, E. J., and R. Morice.** 1991. Miliary pulmonary infection caused by *Mycobacterium terrae*. Chest **100**:1449–1450.
33. **Peterson, G. L.** 1983. Determination of total protein. Methods Enzymol. **91**:95–119.
34. **Power, E. G. M., and A. D. Russell.** 1988. Assessment of 'Cold Sterilog Glutaraldehyde Monitor.' J. Hosp. Infect. **11**:376–380.
35. **Rehork, B., and S. Azzaui.** 1991. Protein load and effectiveness of instrument disinfectants at standing times of several days under practice conditions. Zentralbl. Hyg. Umweltmed. **192**: 177.
36. **Robinson, R. A., H. L. Bodily, D. F. Robinson, and R. P. Christensen.** 1988. A suspension method to determine reuse life of chemical disinfectants during clinical use. Appl. Environ. Microbiol. **54**:158–164.
37. **Rosenblum, L. S., M. E. Villarino, O. V. Nainan, M. E. Melish, S. C. Hadler, P. P. Pinsky, W. R. Jarvis, C. E. Ott, and H. S. Margolis.** 1991. Hepatitis A outbreak in a neonatal intensive care unit: risk factors for transmission and evidence of prolonged viral excretion among preterm infants. J. Infect. Dis. **16**:476–482.
38. **Rutala, W.** 1990. APIC guidelines for the selection and use of disinfectants. Am. J. Infect. Control **18**:99–117.
39. **Rutala, W. A., E. P. Clontz, D. J. Weber, and K. K. Hoffmann.** 1991. Disinfection practices for endoscopes and other semicritical items. Infect. Control Hosp. Epidemiol. **12**:282–288.
40. **Spach, D. H., F. E. Silverstein, and W. E. Stamm.** 1993. Transmission of infection by gastrointestinal endoscopy and bronchoscopy. Ann. Intern. Med. **118**:117–128.
41. **Tandon, R. K.** 1991. Endoscopic disinfection: practices and recommendations. J. Gastroenterol. Hepatol. **6**:37–39.
42. **Tyler, R., G. A. J. Ayliffe, and C. Bradley.** 1990. Virucidal activity of disinfectants: studies with the poliovirus. J. Hosp. Infect. **15**:339–345.
43. **Van Gossum, A., M. Loriers, E. Serruys, and M. Cremer.** 1989. Methods of disinfecting endoscopic material: results of an international survey. Endoscopy **21**:247–250.
44. **Wayne, L. G., and G. P. Kubica.** 1986. Mycobacteriaceae, p. 1436–1457. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
45. **Whyman, C. A., S. A. McDonald, and D. Zoutman.** 1991. Unsuspected dilution of glutaraldehyde in an automatic washer for flexible fibreoptic endoscopes. Can. J. Infect. Control **6**:91–93.
46. **Wolinsky, E.** 1992. Mycobacterial diseases other than tuberculosis. Clin. Infect. Dis. **15**:1–12.