Elimination of high titre HIV from fibreoptic endoscopes

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Abstract

Concern about contamination of fibreoptic endoscopes with human immunodeficiency virus (HIV) has generated a variety of disruptive and possibly unnecessary infection control practices in endoscopy units. Current recommendations on the cleaning and disinfection of endoscopes have been formulated without applied experimental evidence of the effective removal of HIV from endoscopes. To study the kinetics of elimination of HIV from endoscope surfaces, we artificially contaminated the suction-biopsy channels of five Olympus GIF XQ20 endoscopes with high titre HIV in serum. The air and water channels of two instruments were similarly contaminated. Contamination was measured by irrigating channels with viral culture medium and collecting 3 ml at the distal end for antigen immunoassay. Endoscopes were then cleaned manually in neutral detergent according to the manufacturer's recommendations and disinfected in 2% alkaline glutaraldehyde (Cidex, Surgikos) for two, four, and ten minutes. Contamination with HIV antigens was measured before and after cleaning and after each period of disinfection. Initial contamination comprised 4.8×10^4 to 3.5×10^6 pg HIV antigen/ml. Cleaning in detergent achieved a reduction to 165 pg/ml (99.93%) on one endoscope and to undetectable levels (100%) on four. After two minutes in alkaline glutaraldehyde all samples were negative and remained negative after the longer disinfection times. Air and water channels, where contaminated, were tested after 10 minutes' disinfection and were negative. These findings underline the importance of cleaning in removing HIV from endoscopes and indicate that the use of dedicated equipment and long disinfection times are unnecessary.

Concern about the possible transmission of the human immunodeficiency virus (HIV) during endoscopy has led many units to adopt long disinfection times for endoscopes used on infected patients. Furthermore, some endoscopists reserve equipment solely for use on such patients. Although these practices are disruptive and thought to be unnecessary,^{1,2} there is no experimental evidence of the extent to which HIV is removed from endoscope surfaces by cleaning and disinfection.

Extrapolation from *in vitro* tests of disinfectant activity to clinical practice is unreliable. Disinfectants tested against HIV in suspension may be less effective against HIV dried on a surface.³ The type of surface to be disinfected, the presence of organic matter, and the effect of

precleaning influence the disinfection of equipment.⁴⁻⁶

Recommendations for the decontamination of equipment should be based on applied quantitative experiments on the effects of cleaning and timed exposure to disinfectant. Contamination with HIV has been found on one third of endoscopes used on patients with AIDS but in amounts that are too small to quantify accurately.⁷ We have therefore contaminated endoscopes artificially with high titre HIV in serum to measure the effects of cleaning and disinfection.

Methods

VIRUS INOCULUM

HIV 1 (RF variant) was boosted by co-cultivation of infected and uninfected T cell lines in a ratio of 1:10. Culture fluid was harvested after seven days, clarified by centrifugation, and stored in liquid nitrogen. The titre of HIV antigen was measured by enzyme linked immunosorbent assay (Abbott Diagnostic Products, Germany) and expressed in pg antigen/ml. Viral infectivity was confirmed by observation of syncytium formation in C8166 cell cultures inoculated with a 10^{-4} dilution of the virus stock. At the time of use virus was thawed and 1 ml was added to 3 ml fetal calf serum (for endoscopes 1 and 2) and 3 ml was added to 1 ml of fetal calf serum (endoscopes 3, 4, and 5).

ENDOSCOPE CONTAMINATION

Application of the virus to the endoscope, washing, and disinfection were performed under class 3 containment facilities. An Olympus GIF XQ20 endoscope (kindly provided by Keymed) was used for the experiment which was performed on five separate occasions. Four ml serum infected with HIV ('the inoculum') was aspirated by syringe through the channels of the endoscope and the residue discarded. On two occasions all the internal surfaces of the endoscope were contaminated by aspiration of the inoculum into the suction-biopsy, air, and water channels. On the other three occasions the whole inoculum was aspirated into the suction-biopsy channel to provide a higher level of initial contamination in one site. The endoscope was allowed to stand for three minutes then the amount of contamination was measured by sampling in the manner described below.

SAMPLING

The channels were sampled by irrigation with 30 ml RPMI 1640 viral medium containing 10% fetal calf serum, 1% penicillin, streptomycin,

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Accepted for publication
29 August 1989

and L-glutamine. Medium was flushed through the suction port with the suction valve depressed and 3 ml collected at the distal end. Samples were diluted by serial 10-fold reductions to 10⁻⁶ to allow high titre virus to be quantified using the Abbott antigen immunoassay which is calibrated over a range of 30–1000 pg/ml.

After sampling the endoscope was immersed in 0.5% detergent (Neutral Detergent, Applied Chemicals, Coventry) and the valves were removed. The insertion tube was wiped with gauze and the suction-biopsy channel was brushed through twice. The valves, valve housings, suction port, and instrument tip were brushed thoroughly and all channels were irrigated with detergent using a syringe attached to the all-channel irrigation adapter. The endoscope was then reassembled, rinsed with sterile water, and resampled.

The endoscope was disinfected by aspirating freshly activated 2% alkaline glutaraldehyde (Cidex, Surgikos) into the contaminated channels. The suction-biopsy channel was flushed with sterile water after two minutes' disinfection and resampled. Further disinfectant was then aspirated to give a cumulative exposure to disinfectant of 10 minutes. Sampling was repeated after four minutes' and 10 minutes' disinfection. Because of the practical difficulty of sampling the air and water channels under the conditions of the experiment these channels were only sampled after 10 minutes' disinfection.

CONTROLS

(1) To confirm that sampling by irrigation did not cause loss of virus due to carry over of disinfectant, an uncontaminated endoscope was immersed in 2% alkaline glutaraldehyde then rinsed and sampled as above. 100 µl of virus stock was added directly to the sample which was then inoculated into tissue cultures. Viability of the virus was shown by cell fusion and the formation of syncytia.

(2) 250 pg HIV antigen lysate was added to three irrigation samples and to three controls of buffered saline. There was no difference between the antigen titre of irrigation samples and controls, confirming that the assay was not affected by residual traces of glutaraldehyde in irrigation samples.

Results

The antigen titre of the inoculum used for the first two endoscopes was 2.5 × 10⁶ pg/ml and 7.5 × 10⁶ pg/ml for the last three. Sampling by

irrigation retrieved 3% of the inoculum (mean of 6.9 × 10⁴ pg/ml for the first two endoscopes and 2.35 × 10⁵ pg/ml for the last three). Irrigation after washing produced samples from four endoscopes which were free of all detectable HIV antigen (<30 pg/ml); samples from the fifth endoscope after washing contained 165 pg/ml of antigen (Table). Thus cleaning achieved a 99.93% reduction in detectable HIV antigen on one endoscope and a mean reduction of at least 99.97% on the remaining four (range 99.94 to 99.99%). All samples were negative after the endoscopes had been disinfected for two minutes in glutaraldehyde.

Discussion

Guidelines for the control of infection in endoscopy units have been formulated without the benefit of relevant experimental data on the inactivation of HIV.^{1,2,8} It is not possible to extrapolate from the in vitro data currently available to clinical practice without introducing speculation. This uncertainty has led to various infection control policies which often recommend a disinfection time of several hours to ensure an adequate margin of safety against HIV.^{8,9} Recommendations based on short disinfection times may fail to gain acceptance without a clear demonstration that they are safe.

Organic protein, precleaning, and the type of surface to be disinfected all influence disinfectant activity.^{4,6} We attempted to reproduce the clinical situation as closely as possible. The amount of HIV on an endoscope used on a patient with AIDS is too small to be measured by antigen immunoassay and has only been detected after amplification using the polymerase chain reaction.⁷ However, in order to measure reductions in viral contamination we used a viral inoculum with an antigen titre approximately 1000 times greater than that likely to be encountered in patients' serum.

One disadvantage of studying disinfection on surfaces is that surviving organisms can only be detected by indirect means – for example, by irrigating or brushing the surface. In this study irrigation retrieved only 3% (95% confidence interval 2 to 5.8) of the inoculum, but we found that brushing produced lower yields. The inability to detect virus in samples therefore does not by itself indicate that the endoscope is free of virus. Comparisons can be made between samples, however, and these showed that cleaning achieved a mean 4.74 log reduction in HIV antigen. This compared favourably with the 4 log reduction which is accepted as the criterion for virucidal efficacy of disinfectants.^{10,11}

Because of the limitations of sampling, the point at which all virus was removed from the endoscopes can only be determined with knowledge of the susceptibility of HIV to glutaraldehyde. Previous studies have shown that two minutes' exposure to 2% alkaline glutaraldehyde causes a 4 log reduction in the titre of HIV.² This suggested that the consecutive reductions achieved with cleaning and two minutes' disinfection in glutaraldehyde effectively decontaminated all five endoscopes. Cleaning followed by disinfection for four minutes in glutaraldehyde

HIV antigens (pg/ml) in samples from five endoscopes contaminated in vitro with HIV in serum

Channels	Before cleaning	After cleaning	After disinfection for:		
			2 min	4 min	10 min
Suction/biopsy	9 × 10 ⁴	Neg	Neg	Neg	Neg
Air	–	–	–	–	Neg
Water	–	–	–	–	Neg
Suction/biopsy	4.8 × 10 ⁴	Neg	Neg	Neg	Neg
Air	–	–	–	–	Neg
Water	–	–	–	–	Neg
Suction/biopsy	3.5 × 10 ⁵	Neg	Neg	Neg	Neg
Suction/biopsy	1.2 × 10 ⁵	Neg	Neg	Neg	Neg
Suction/biopsy	2.35 × 10 ⁵	165	Neg	Neg	Neg

hyde, as recommended by a Working Party on AIDS of the British Society of Gastroenterology,² would therefore incorporate a reasonable margin of safety against HIV.

Cleaning in detergent was clearly extremely effective in removing HIV and serum from endoscopes. (This result cannot necessarily be extrapolated to other micro-organisms such as *Mycobacteria* or hepatitis B virus. These are the subject of further studies which we hope to publish shortly.) Disinfectants may fail if organic material is not first removed by cleaning.^{3,12} This study showed that cleaning can be performed effectively by hand, but the use of an automated machine ensures that the procedure is always performed in full and reduces the exposure of staff to infected material.

We wish to thank the Department of Health, Medical Research Council, Keymed, Surgikos, and the Sanatorium Society for financial support.

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