A quantitative assessment of residual protein levels on dental instruments reprocessed by manual, ultrasonic and automated cleaning methods

M. Vassey,[1] C. Budge,[2] T. Poolman,[3] P. Jones,[4] D. Perrett,[5] N. Nayuni,[6] P. Bennett,[7] P. Groves,[8] A. Smith,[9] M. Fulford,[10] P. D. Marsh,[11] J. T. Walker,[12] J. M. Sutton[13] and N. D. H. Raven[14]

IN BRIEF

- Informs the reader about the efficacy of different types of dental instrument cleaning process.
- Defines for the first time the relative efficacy of these cleaning processes as used in real-life dental practice settings.
- Highlights differences between the cleanability of different types of dental instruments.
- Aids in understanding the potential risk of transmitting variant CJD between patients.

RESEARCH

Objective To assess residual protein on dental instruments cleaned in general dental practice by manual, manual plus ultrasonic and automated washer disinfector (AWD) processes. **Design and setting** Instruments submitted by 30 dental surgeries in the South West of England. **Subjects (materials) and methods** Instruments analysed were matrix bands, associated retaining clips, diamond and stainless steel burs, extraction forceps and hand scalers. Each instrument was visually assessed under magnification for residual debris. Residual protein was extracted by immersion in detergent and sonication. A collection of used but uncleaned instruments of each type (n = 177) was also analysed for adherent protein using ophthalaldehyde/N–acetylcysteine reagent. **Main outcome measures** Residual protein levels allowed comparisons to be made on the effectiveness of different cleaning processes. **Results** One thousand, three hundred and four instruments were analysed. Observational data demonstrated several shortcomings in cleaning chemistries and operation of the AWD. For uncleaned instruments, median residual protein levels ranged from 0.4 μg (stainless steel burs) to 462 μg (extraction forceps). Following manual washing, median protein levels ranged from 0.3-78 μg; for manual plus ultrasonic washing, levels ranged from 9-39 μg and AWD levels ranged from 0.3-27 μg. Manual washing combined with ultrasonic cleaning was significantly less effective than the other two processes ($p < 0.008$). AWDs reduced the variability in the cleaning process. No correlation was found between visual scoring and residual protein determination. **Conclusion(s)** There was a wide variation in residual protein levels both within and between different methods and instruments and this underlines the complexity of this process.

INTRODUCTION

A number of concerns have been raised over the efficacy of instrument decontamination in sterile services departments,[1,2] endoscopy reprocessing units[3,4] and general dental practice.[5] A critical control point within the decontamination cycle is the efficacy of the cleaning process. A large observational study has demonstrated that in general dental practice the cleaning of dental instruments is poorly controlled[6] and insufficiently managed.[7] For some dental devices that are difficult to clean, such as matrix bands and endodontic files, this may result in visible blood contamination remaining even after reprocessing,[8,9] with consequent recommendations for these items to be classed as single use only.[10,11] In addition to blood contamination, concerns have been raised that the proteinaceous residues derived from previously treated patients may represent a risk of transmission of vCJD.[12] These concerns are heightened by reduced susceptibility of the infectious agent to be removed and/or inactivated by conventional cleaning and sterilisation processes.[13]

Within general dental practice the level of risk of cross-infection associated with poor instrument decontamination is unclear. Anecdotal evidence suggests that transmission of viruses such as hepatitis B[14] and bacteria such as *Staphylococcus aureus* can occur in dental practice.[15–17] Since no systematic surveillance of post-operative infections following dental procedures is undertaken, it is unclear how frequently these events take place in reality. It would seem prudent, therefore, that the reprocessing of dental instruments follows the route used in the reprocessing of other surgical instruments identified in European standards[18–20] and national guidelines[21,22] and under the management of an appropriate quality management system.[23]

While a number of studies have been undertaken of instruments reprocessed in sterile service departments,[24–26] little work has concentrated on the range of processes used to clean commonly used dental instruments, particularly in a real-life setting. The aim of this study was to investigate the efficacy of cleaning dental instruments by measuring residual protein following a) manual cleaning

[1,3]Project Team Leader, [2,4]Assay Scientist, [11]Programme Leader, TB & Public Health Microbiology Programme, [12]Senior Project Team Leader, [13]*Scientific Leader - Healthcare Biotechnology, [14]General Project Manager, Centre for Emergency Preparedness and Response, Health Protection Agency, Porton Down, Salisbury, SP4 0JG; [5]Professor of Bioanalytical Science, [6]Project Scientist, William Harvey Research Institute, Barts & The London School of Medicine and Dentistry, Queen Mary, University of London, John Vane Building, Charterhouse Square, London, EC1M 6BQ; [7]Head of Analysis, Health Protection, [8]Senior Principal Analyst, Department of Health, Wellington House, London; [9]Professor & Consultant Microbiologist, College of Medical, Veterinary & Life Sciences, Glasgow Dental Hospital & School, 378 Sauchiehall Street, Glasgow G2 3JZ; [10]General Dental Practitioner (retired), Shepton Mallet, [11]Professor of Oral Microbiology, School of Dentistry, Leeds Dental Institute, University of Leeds, Clarendon Way, Leeds, LS2 9LU
*Correspondence to: Dr Mark Sutton
Email: mark.sutton@hpa.org.uk
Tel: +44 (0) 1980 612 643

Refereed Paper
Accepted 28 July 2010
DOI: 10.1038/sj.bdj.2011.144

Table 1 Recovery of total protein per instrument per cleaning process. Values expressed as the median total μg of extractable protein per instrument (interquartile range). *Values below the measurable range of the assay were reported as 50% of the Limit of Quantification (LOQ)

Instrument type	Process type: median level of protein detected (25th and 75th percentile), μg per instrument (number of instruments)			
	Uncleaned	Manual	Manual and USB	AWD
Scaler	28.0 (2, 103) (n = 21)	16.1 (6, 34) (n = 80)	30.0 (10, 166) (n = 72)	1.4 (0.3, 16.85) (n = 111)
Matrix band retaining clip	88.0 (47, 116) (n = 30)	78.2 (42, 123) (n = 54)	24.0 (0.3, 107) (n = 68)	11.9 (0.8, 40) (n = 96)
Matrix band	143.0 (52, 312) (n = 31)	1.1 (0.3, 65) (n = 38)	17.9 (0.3, 99) (n = 56)	0.3* (0.3, 50) (n = 72)
Extraction forceps	462.0 (285, 759) (n = 31)	0.3* (0.3, 38) (n = 64)	38.8 (12, 100) (n = 69)	27.0 (2, 100) (n = 109)
Steel bur	0.4 (0.3, 4) (n = 33)	5.0 (0.3, 10) (n = 75)	9.0 (4, 19) (n = 77)	10.1 (7, 12) (n = 22)
Diamond bur	0.6 (0.3,2) (n = 31)	2.65 (0.6, 8) (n = 76)	9.6 (6, 20) (n = 63)	0.3* (0.3, 4) (n = 102)

only, b) manual plus ultrasonic cleaning, and c) automated washer disinfector (AWD) cleaning undertaken as part of the routine dental surgery reprocessing schedule in dental practices.

METHODS

Selection of dental surgeries

Dentists located in the South West of England were selected from local primary care trusts' published lists of practices holding NHS contracts. The surgeries were contacted in writing inviting them to participate in a dental instrument decontamination study. If practitioners wished to take part in the study they were asked to indicate which instrument cleaning process was used in their surgery: manual cleaning only, manual plus ultrasonic cleaning or automated washer disinfector (AWD). From this initial list, ten surgeries from each instrument cleaning group were randomly selected to provide instruments for analysis. No instruments submitted for analysis in this study were returned to the surgeries; all were replaced with new items funded by the study. No information was available regarding either the instrument or the number of times each instrument had been used and reprocessed. Instruments were collected over the period December 2005 to October 2007.

Selection of dental instruments

Six different types of dental instrument, selected to represent a range of complex surfaces and degree of invasiveness, were analysed from each surgery. The instruments were extraction forceps, sickle scalers, diamond and steel burs, matrix bands (Siqveland) and the associated matrix band retaining clips.

In order to provide a source of reference for the extent of protein removal following each cleaning process, a collection of used but uncleaned instruments was also assayed for protein content. These instruments were obtained from a similar cohort of dental surgeries in the South West of England. All these instruments were steam sterilised through a 134°C cycle before analysis to enable safe handling.

Decontamination equipment and process data collection

For each dental surgery, an observer collected information by direct observation of the cleaning process and equipment and, where appropriate, reviewed relevant documentation on a standardised data collection form.[5]

Visual assessment of cleaned instruments

All instruments were assessed for visual contamination under a binocular microscope and scored between 0 (no visible debris) and 3 (high levels of visible debris) at HPA, CEPR, by three independent operators. The scoring system was based on that previously used and published[9] for the visual scoring of endodontic files. Instruments were viewed through an Olympus SZ40 microscope and captured on a Nikon D50 Digital SLR camera with macro lens (SIGMA 50mm 1:2.8 DG MACRO D). Captured images and scores were stored using an image database system (Image Access Standard 5).

Protein analysis

Residual protein on uncleaned and cleaned instruments was extracted by sonicating the working end of each instrument at 32-38 kHz for 2x60 minutes in freshly prepared 0.05% aqueous Decon 90 (Decon Laboratories Ltd, Sussex, UK) at room temperature using a Medisafe digital PC ultrasonic bath and an Ultrawave model QS3. The wash liquids were assayed for protein concentration using the *o*-Phthalaldehyde/N-acetylcysteine assay as previously described[27] with a limit of quantification of 0.3 μg of protein per instrument. The protein values from both washes were combined to obtain the total residual protein on each instrument. Instruments with two working ends were sonicated twice in the same wash solution.

Statistical analysis

Continuous data were expressed as median and interquartile ranges (IQR). Two proportion and Mann-Whitney pairwise analysis was used to compare the significance of relative residual protein levels between processes ($p < 0.008$ with a Bonferroni correction applied) using MiniTab (version 15). Spearman's rank correlation coefficient (rho) and ROC curve regression models were both applied independently to assess the correlation of visual score and corresponding residual protein level.

RESULTS

Decontamination equipment and processes used

Where manual cleaning of instruments was observed, 7/14 surgeries used no detergent and 7/14 used surgical handwash. Where ultrasonic cleaning of instruments was reported, the detergent used in the ultrasonic bath was either neutral detergent or the manufacturer's recommended brand in 4/8 sites. The remaining 4/8 sites used a disinfectant/detergent combination. In surgeries undertaking ultrasonic cleaning of instruments the range of time for emptying the ultrasonic bath varied from three to

40 hours. In none of the eight surgeries was the ultrasonic bath subjected to any cleaning or ultrasonic efficacy testing.

The machine in all ten surgeries operating AWDs was from the same manufacturer (Medisafe), with the same model (Pico), using the same programme and the same detergent (3E-Zyme). The automated cleaning process was by spray action only; no channel irrigation of lumened devices (handpieces) was observed. No surgery undertook checks to ensure correct loading of each carrier before processing and no records of cleanliness failures were kept. A small number of surgeries (3/10) had undertaken some form of performance testing of the AWDs. There was insufficient information available at the surgeries to determine whether any machine had been tested for cleaning efficacy. No test records had been independently audited by an Authorised Person (Sterilisers) for any surgeries using an AWD.

Visual assessment

The median visual scores across all the different instrument types for the range of process used were as follows: manual only (0.5), manual plus ultrasonic (0.25) and AWD (0.25). The median visual scores for each type of instrument for all cleaning processes showed that the steel burs scored highest (0.5) followed by the forceps (0.25), matrix band retaining clips (0.25), matrix bands (0.25), diamond burs (0.25), with scalers having the lowest median visual score of 0.

The maximum median visual score seen for any instrument type across the different cleaning processes was 0.75; this was observed on matrix band retaining clips cleaned by a manual process only, and steel burs cleaned by a manual plus an ultrasonic process and also by AWD.

Effect of cleaning on instrument residual protein levels

For all instruments subjected to a cleaning process (n = 1,304), 72% had detectable residual protein contamination. The median amount across all the instruments tested was 10.25 μg with a range from 0.3 μg to 3.85 mg. To provide an estimate of the protein load on instruments before cleaning, 177 used instruments were analysed and found to have a wide range in level of protein contamination (Table 1). The forceps had the highest median values of 462 μg (interquartile range of 285–759 μg), with the diamond and steel burs having the lowest median protein recovered: 0.6 μg (0.1–2 μg) and 0.4 μg (0–3 μg) respectively.

Analysis of the effect of the three cleaning processes on the six different types of instrument is shown in Table 1. Results varied both within and between the different processes and instrument types. Compared to uncleaned sickle scalers, the median residual protein levels on cleaned sickle scalers using manual or manual and ultrasonic cleaning were higher. This phenomenon was also observed for steel and diamond burs with the exception of diamond burs in the AWD. For the matrix bands and matrix band retaining clips, the lowest level of residual protein was found in those cleaned in the AWD. The lowest levels of residual protein from extraction forceps was found in those subjected to a manual cleaning process.

Statistically, the recovery of residual protein from the combined manual and ultrasonic process across all instrument types was significantly higher than the other two processes ($p < 0.008$). Although use of an AWD was not statistically better overall than manual cleaning alone, the automated method did significantly reduce the number of instruments with residual protein levels above 50 μg per instrument (Figs 1a–c).

Effect of instrument type on cleanability

Comparison of the residual protein contamination following cleaning of all instrument types demonstrated that the extraction forceps had the highest median level of residual protein of 28.4 μg (IQR 5–84 μg). This was followed by the matrix band retaining clip, scalers, steel burs and matrix bands.

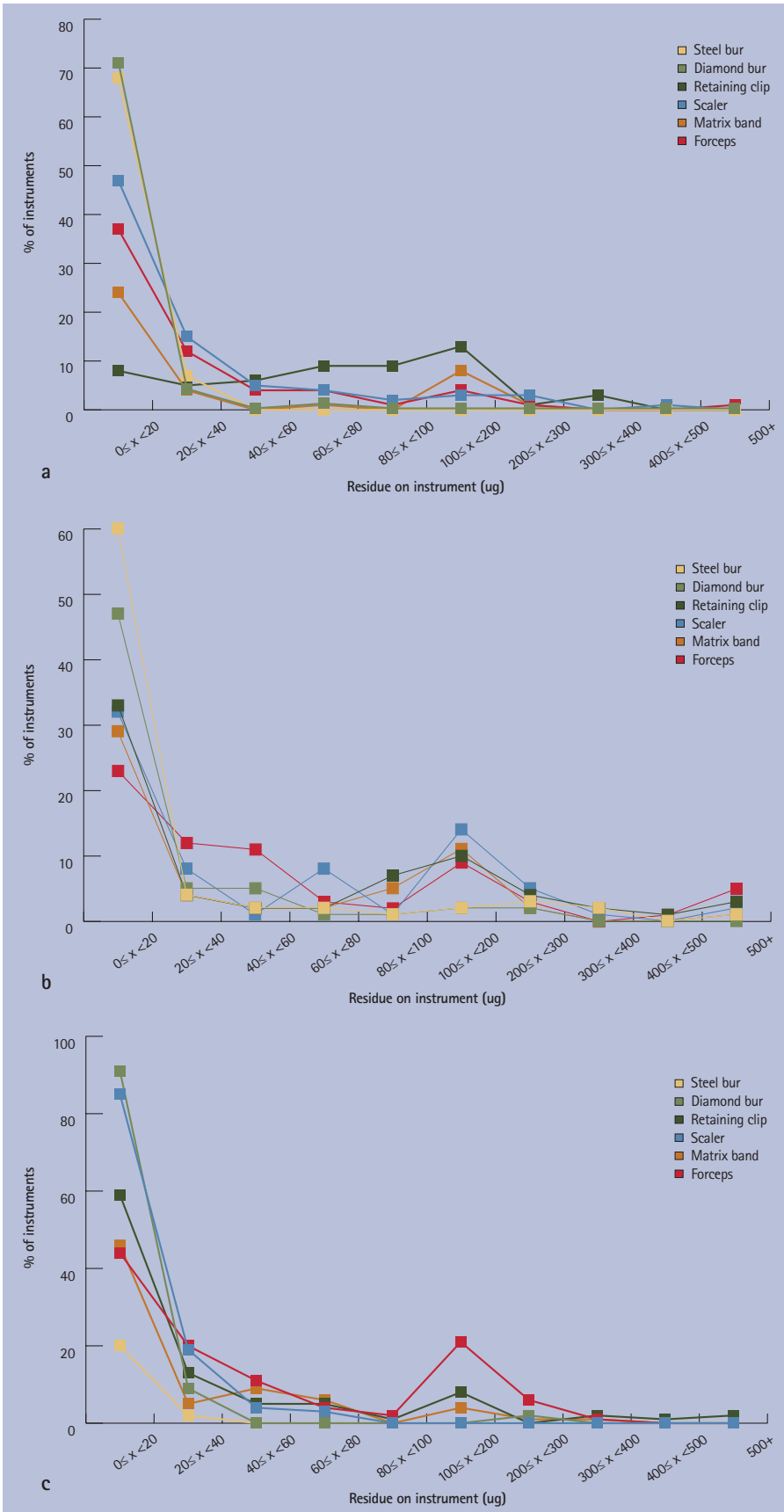
Correlation between visual scoring and protein contamination

Comparative analysis of the visual score data with residual protein data showed no correlation as determined by a Spearman's rho analysis. All correlations between processes and instruments showed r-values of less than 0.7, meaning no correlation between the visual score assigned by the operator and the actual residual protein present on the instrument. This was regardless of process used or instrument type.

DISCUSSION

This study is the first to provide detailed analytical findings on the efficacy of manual and automated cleaning processes carried out under general dental practice conditions on a large number of dental instruments. Observations on the processes, cleaning chemicals and management control are similar to earlier reports from a larger observational study[6] demonstrating multiple shortcomings. Our findings add to the literature by providing data on AWDs, which before 2005 were not in widespread use in dental practice in the UK. These observational data are important when interpreting the results of our study since the efficacy of the cleaning process will be strongly influenced by a number of interrelated factors such as cleaning chemicals, water quality, physical energy used (manual, ultrasonic, water jets), cleaning time, cleaning temperature, instrument set-up and design. The manual plus ultrasonic method was significantly less efficient than the other processes as determined by Mann-Whitney statistical test ($p < 0.008$ with a Bonferroni correction applied). The use of inappropriate cleaning chemicals, for example, Hibiscrub, would have compromised the cleaning process. Similarly, failure to change the ultrasonic bath water frequently allows the build-up of contaminants that will increase detectable protein residues on instruments exposed to these liquids as opposed to reducing them. These observations may explain the higher levels of protein found on instruments compared to uncleaned instruments following the use of ultrasonic baths. Additionally, the efficacy of the ultrasonic baths may have been compromised since no periodic testing of functionality of these devices was undertaken. Similarly, further improvements in the cleaning efficacy of the AWDs beyond the level seen in this study are likely if equipment is validated and tested according to the required European standards. The differences in protein levels on forceps processed by manual-only cleaning and in the AWD may be explained by incorrect loading of the AWD, since no checks were made on the AWD loading pattern. The use of an enzymatic detergent (3E-zyme) containing protein may have contributed to the residual protein measurements for the AWD samples, if inadequately rinsed. Nevertheless, use of an AWD gave the lowest median levels of residual protein for four of the six instrument types.

Analysis of residual protein levels on the different instrument types cleaned by the



Figs 1a, b, c Bar charts showing the distribution of protein residue measurements for different instruments and cleaning processes. The protein residues for instruments cleaned by manual only (a), manual with ultrasonic (b) or automated washer-disinfector (c) were measured. Data, expressed as μg of protein per instrument, is provided to demonstrate the greater distribution of data at the lower end of the measurable range (shown in 20 μg classes) and to provide an indication of the distribution of measurements at the higher ranges

three processes demonstrates the innate complexities of cleaning dental instruments. Under general dental practice conditions, no single process was universally most effective at cleaning all instrument types. In addition, our data showed that there was no single instrument type which proved to have consistently either the highest or lowest levels of residual protein following cleaning. Manual cleaning of dental instruments is subjective and not reproducible, with variations, for example, in the type of detergent, water temperature, brush type, and in the number and strength of strokes used in the cleaning process, with minimal opportunity for control or validation. However, when carried out as a sole method of cleaning for some types of instrument, the process can be highly effective as demonstrated by the low levels of protein recovered from forceps (0.3 μg, IQR 0.3–38 μg). This may well reflect the targeted cleaning of joints and serrations by a dedicated staff member when compared to forceps which may have been incorrectly loaded into an AWD that has not been validated.

Manual cleaning was not as effective for the remaining instrument types although overall statistical analysis does not demonstrate a significant difference compared to the AWD used under the conditions in this study. Proportional analysis of the four larger instrument types (scalers, matrix bands, retaining clips and forceps) demonstrated that use of the AWD showed a significantly greater number of instruments with <50 μg protein recovered than the manual process ($p < 0.118$). This difference may well be enhanced further in inappropriately installed, tested and operated equipment. It should be noted that this study analysed the effect of cleaning in only one model of AWD using the detergent and wash cycle as specified by the manufacturer. Other models of AWD, chemistries and cleaning cycle parameters may produce different results in terms of cleaning efficacy.

The lack of correlation between visual scoring systems and protein residues has been reported previously,[9,24–26] demonstrating that visual assessment even under high magnification has a low predictive value for determining residual protein levels. However, the examination of cleaned instruments under magnification and controlled lighting is highly specific in detecting gross soil deposits and material defects in

instruments and forms an important part of overall instrument decontamination quality control. Further work is necessary to develop tests that are more sensitive and specific for determining a quantifiable end point in the cleaning process and which can be used in general dental practice.

The ortho-phthaldialdehyde assay only reacts with exposed primary amines on proteins and there are no data currently available that correlate the values detected on instruments post-cleaning in this study with potential infectivity from TSE infectious agents,[28] although it does provide useful data for assumptions on the likely reduction in risk through improvements in cleaning processes.[29] Our findings are also useful to put other instruments and processes into context; for example, using a similar methodology, the median residual protein value on cleaned endodontic files was 5.4 μg per file (range 0.5-63.2 μg).[27] For other surgical specialities it has been demonstrated that protein residue levels in the range 0.1 μg to >1.0 mg per instrument are not uncommon.[24–26]

No threshold value has been defined as representing an acceptable residual level of protein on any form of surgical instrument after reprocessing, with guidance provided as to achieving 'best practice' rather than specific performance levels. Further work should focus on defining achievable baseline levels of residual protein on surgical and dental instruments, for different cleaning processes. This could form the basis for improved decontamination practices. Such a threshold would enable policy decisions to be made on which of the alternative cleaning processes would remain part of acceptable practice and drive improvements to instrument installation, commissioning and routine use. In particular, the use of ultrasonic baths needs to be reviewed and improved guidelines established. While the expectation is that improved procedures would result in reduced levels of residual protein contamination, there is little evidence in the literature to support this conclusion and further studies would be invaluable.

The findings described here are especially relevant since the instruments were reprocessed using cleaning practices in common use and were compared to instruments cleaned in AWDs. Despite inadequate set-up and use, the AWDs demonstrated improved consistency in the cleaning of instruments. No doubt further improvements in efficacy could be made with closer attention to the relevant standards and guidelines for their operation and management. Some instruments, such as extraction forceps, may benefit from a manual clean before loading into an AWD. Further improvements in cleaning efficacy could also be made with appropriate education and training of dental staff in cleaning parameters. Practitioners should also receive more logistical support to assist with the technical challenges posed by inadequate commissioning and testing of equipment by manufacturers and suppliers.

In conclusion, this study provides additional data from large numbers of dental instruments on the levels of residual protein following cleaning in general dental practice. The study highlights areas where improvements in cleaning efficacy should be achievable with enhanced training and technical support for equipment installation, operation and routine testing.

Funding for the authors' research was received from the Department of Health. The views expressed in the publication are those of the authors and not necessarily those of the Department of Health or the Health Protection Agency.

1. Report of a Scottish Executive Health Department Working Group. *The decontamination of surgical instruments and other medical devices.* 2001. http://www.sehd.scot.nhs.uk/publications/hdl30101annex.pdf.
2. NHS Estates. *Decontamination review: report on a survey of current decontamination practices in healthcare premises in England.* 2001. http://www.dh.gov.uk/PublicationsAndStatistics/Publications/PublicationsPolicyAndGuidance/PublicationsPolicyAndGuidanceArticle/fs/en?CONTENT_ID=4120912&chk=lqhPNX.
3. Ramakrishna B S. Safety of technology: infection control standards in endoscopy. *J Gastroenterol Hepatol* 2002; **17:** 361–368.
4. Department of Health, Social Services and Public Safety (Belfast). *The report of an independent review of endoscope decontamination in Northern Ireland by Northern Ireland.* 2004. www.dhsspsni.gov.uk/endoscope-report.pdf.
5. NHS Scotland Sterile Services Provision Review Group. *Survey of decontamination in general dental practice.* 2004. http://www.scotland.gov.uk/Resource/Doc/26800/0012665.pdf.
6. Bagg J, Smith A J, Hurrell D, McHugh S, Irvine G. Pre-sterilisation cleaning of re-usable instruments in general dental practice. *Br Dent J* 2007; **202:** E22.
7. Smith A J, Creanor S, Hurrell D, Bagg J, McGowan M. Management of infection control in dental practice. *J Hosp Infect* 2009; **71:** 353–358.
8. Lowe A H, Bagg J, Burke F J T, MacKenzie D, McHugh S. A study of blood contamination of Siqveland matrix bands. *Br Dent J* 2002; **192:** 43–45.
9. Letters S, Smith A J, McHugh S, Bagg J. A study of visual and blood contamination on reprocessed endodontic files from general dental practice. *Br Dent J* 2005; **199:** 522–525.
10. Burns H, Watkins R. *Letter to Dentists, General Dental Service, Community Dental Service, Hospital Dental Service. Important advice for dentists on re-use of endodontic instruments and variant Creutzfeldt-Jakob Disease (vCJD).* 2007. http://www.sehd.scot.nhs.uk/cmo/CMO(2007)05.pdf.
11. Cockcroft B. Advice for dentists on re-use of endodontic instruments and variant Creutzfeldt-Jakob Disease (vCJD). 2007. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/documents/digitalasset/dh_074000.pdf.
12. Department of Health, HPIHSD Analytical Team. *Potential vCJD transmission risks via dentistry: an interim review.* 2007. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_081218.pdf.
13. Taylor D M. Inactivation of prions by physical and chemical means. *J Hosp Infect* 1999; **43 (Suppl):** S69–S76.
14. Redd J T, Baumbach J, Kohn W, Nainan O, Khristov M, Williams I. Patient-to-patient transmission of hepatitis B virus associated with oral surgery. *J Infect Dis* 2007; **195:** 1311–1314.
15. Martin M V, Hardy P. Two cases of oral infection by Methicillin resistant *Staphylococcus aureus. Br Dent J* 1991; **170:** 63–64.
16. Kurita H, Kurashina K, Honda T. Nosocomial transmission of methicillin resistant *Staphylococcus aureus* via the surfaces of the dental operatory. *Br Dent J* 2006; **201:** 297–300.
17. Rokadiya S, Malden N J. An implant periapical lesion leading to acute osteomyelitis with isolation of *Staphylococcus aureus. Br Dent J.* 2008.
18. British Standards Institution. *BS EN 13060:2004. Small steam sterilizers.* London: BSI, 2004.
19. British Standards Institution. *BS EN ISO 17665-1:2006. Sterilization of health care products. Moist heat. Requirements for the development, validation and routine control of a sterilization process for medical devices.* London: BSI, 2006.
20. British Standards Institution. *BS EN ISO 15883-1:2006. Washer-disinfectors. General requirements, terms and definitions and tests.* London: BSI, 2006.
21. Health Protection Scotland. *Local decontamination units: guidance on the requirements for equipment, facilities and management.* 2007. http://www.documents.hps.scot.nhs.uk/hai/decontamination/publications/ldu-001-02-v1.1.pdf.
22. Department of Health. *Health Technical Memorandum 01–05: Decontamination in primary care dental practices.* 2009. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/documents/digitalasset/dh_112542.pdf.
23. British Standards Institution. *BS EN ISO 13485:2003. Medical devices – quality management systems – requirements for regulatory purposes.* London: BSI, 2003.
24. Lipscomb I P, Sihota A K, Keevil C W. Comparative study of surgical instruments from sterile-service departments for presence of residual gram negative endotoxin and proteinaceous deposits. *J Clin Microbiol* 2006; **44:** 3728–3733.
25. Baxter R L, Baxter H C, Campbell G A *et al.* Quantitative analysis of residual protein contamination on reprocessed surgical instruments. *J Hosp Infect* 2006; **63:** 439–444.
26. Murdoch H, Taylor D, Dickinson J *et al.* Surface decontamination of surgical instruments: an ongoing dilemma. *J Hosp Infect* 2006; **63:** 432–438.
27. Smith A J, Letters S, Lange A, Perrett D, McHugh S, Bagg J. Residual protein levels on reprocessed dental instruments. *J Hosp Infect* 2005; **61:** 237–241.
28. Barron R M, Campbell S L, King D *et al.* High titers of transmissible spongiform encephalopathy infectivity associated with extremely low levels of PrPSc *in vivo. J Biol Chem* 2007; **282:** 35878–35886.
29. Department of Health. *Assessing the risk of vCJD transmission via surgery: an interim review.* 2005. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4113542.pdf.