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Decontamination of orthodontic bands following size determination and cleaning

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Abstract

Objective: To measure the effectiveness of ultrasonic cleaning to decontaminate orthodontic molar bands following size determination using a quantitative antibody capture assay technique.

Design: A prospective, cross-sectional, clinical and laboratory investigation.

Setting: The Orthodontic Department of the Charles Clifford Dental Hospital and the Microbiology Laboratory of the School of Clinical Dentistry, Sheffield.

Participants: 32 patients about to start orthodontic treatment with fixed orthodontic appliances.

Methods: Four first molar bands were tried in the mouth and then removed. They were randomly assigned either for no decontamination (Control) or to be decontaminated in an ultrasonic cleaning bath for 15 minutes (Experimental). The bands were placed in a pre-determined volume of phosphate-buffered saline (PBS) and assayed by Enzyme-Linked Immunosorbent Assay (ELISA) for albumin, to detect the presence of blood and amylase, to detect the presence of saliva.

Results: 50 percent of decontaminated molar bands showed detectable amounts of amylase, albumin or both. The quantity of detectable amylase was significantly reduced on the cleaned compared with uncleaned bands ($p=0.003$), however the reduction in the quantity of albumin was not statistically significant ($p=0.053$).

Conclusions: Ultrasonic cleaning for 15 minutes reduces, but does not always eliminate salivary proteins (amylase) from tried-in bands. It is less effective at removing serum protein (albumin). There is a need, therefore, to investigate effective means of cleaning organic material from orthodontic bands if they are to be adequately sterilized and reused.

Introduction

Preformed stainless steel bands of varying sizes are commonly placed around posterior teeth during fixed appliance treatment. It frequently takes several attempts to achieve the correct size. Orthodontic bands are expensive; therefore it is not financially viable to consider these as single-use, disposable items if they have been tried in the mouth and found to be the wrong size. As a result the practice of re-use and re-circulation is widely accepted and carried out, however the bands will have been contaminated with both blood and saliva during the trying in process.

The decontamination process usually consists of three stages, which are cleaning, sterilization and return to storage[1]. Recommendations for the initial cleaning process include removal of the contaminant by hand, the use of an ultrasonic bath and disinfectant, enzyme-based cleaning solution or instrument washer. This is followed by the use of a steam autoclave as the method of choice for sterilization of all dental instruments. However, if the method of cleaning is inadequate in both quantity and quality then any remaining debris or contaminant may prevent exposure of the surface to the pressurised steam and latent heat. This may result in potentially harmful blood-borne agents or contaminants remaining on the orthodontic band surface.

The design of orthodontic bands and the various welded attachments presents a significant potential for contamination by either blood or saliva during use. For example, capillary action can draw fluids into the buccal tubes used for archwires or other accessories and these sites are very poorly accessible to the cleaning process. These design features then may facilitate the spread of infectious agents between patients.

There is currently little information on the extent of organic contamination and success of cleaning procedures for bands that are removed during the routine fitting of a fixed appliance on a patient, because they are found to be the wrong size. Fulford et al[2] have shown that bands subjected to an enzymatic cleaner/disinfectant and then sterilized using either a

downward displacement or a vacuum cycle autoclave showed no bacterial growth when inoculated into brain heart infusion culture broth and incubated at 37°C for 5 days. They concluded that there is little risk of a cross-infection hazard occurring with the re-use of previously tried-in and decontaminated molar bands. However, such processes can sometimes damage microorganisms sufficiently to preclude their recovery in laboratory culture but not to kill or eliminate them. Furthermore, there have been few assessments of the removal of viruses from orthodontic bands and it is acknowledged that prions are extremely difficult to inactivate by normal means. Thus, a better assessment of the decontamination process would be the removal and/or retention of organic matter.

The effectiveness of the decontamination procedure on blood removal can be assessed by the Kastle-Meyer test, which is widely applied in forensic medicine for the detection of blood. and has been used previously in a hospital orthodontic environment[3] to detect the presence of occult blood. Originally described in 1926 by Glaister[4], the the Kastle-Meyer test is simple, rapid and sensitive However, as applied in previous studies, merely detecting the presence or absence of blood is not a quantitative method and so cannot be used to assess the level of risk from contamination.

The following investigation was carried out to address two research questions:

- What is the level of contamination with blood and saliva of orthodontic molar bands following size determination in the mouth?

- Is ultrasonic cleaning of tried-in bands for 15 minutes sufficient to reduce or remove this level of contamination so that they can be reused?

The aim of the study was to measure the effectiveness of ultrasonic cleaning to decontaminate orthodontic molar bands following size determination using a quantitative antibody capture assay technique.

Materials and Methods

Design

This study was a prospective, cross-sectional, clinical and laboratory investigation. The intervention was decontamination of the molar band using an ultrasonic cleaning bath following sizing in an orthodontic patient.

Samples and Setting

The study was carried out in the Orthodontic Department of the Charles Clifford Dental Hospital and the Microbiology Laboratory of the School of Clinical Dentistry, Sheffield. The investigation was approved by the South Sheffield Research Ethics Committee (Ref Nos 03/189) and informed consent was obtained from all patients taking part.

The sample consisted of 128 stainless steel first molar bands that had been tried-in the mouth of 32 patients. The patients were being fitted with upper and lower fixed appliances, at the start of orthodontic treatment. A band was placed over the first permanent molar in each quadrant. They were removed from the mouth and randomly assigned, using a block randomisation technique, to one of two groups according to the side they were tried-in:

Control	Bands that were been tried-in the mouth, but not cleaned
Experimental	Bands that were tried-in the mouth and cleaned for 15 minutes in an ultrasonic cleaning bath.

A record was made of whether bleeding was visible when the band was removed or not.

Four bands, which had not been placed in the mouth, were randomly chosen from the same band selection tray and analysed to ensure that the bands were not contaminated prior to trying in the mouth.

Specimen Collection and Laboratory Procedures

Following sizing, removal from the mouth and cleaning for those allocated to the Experimental group, the bands were placed in 1ml phosphate-buffered saline (PBS) pH 7.5 in a suitable container at room temperature, left for 15 min and then vortexed to remove as much organic material as possible. The samples were immediately frozen, so they could all be analysed together.

Once the all 128 bands had been collected, the PBS eluates were thawed and assayed by an antibody capture Enzyme-Linked Immunosorbent Assay (ELISA) for albumin, to detect the presence of blood and amylase, to detect the presence of saliva. Anti- human albumin and anti-human amylase (1:10,000 in bicarbonate buffer pH 9.6; Sigma) were coated onto ELISA wells (Corning Costar, High Wycombe, UK) overnight at 4°C. After washing and blocking with 5 percent skimmed milk, suitably diluted samples were placed in wells for 1 hour at 37°C, washed again and probed with biotin-labelled anti-albumin or anti-amylase antibodies (1:10,000). Antibodies were biotin-labelled by reaction with biotin-N-hydroxysuccinimide ester (Sigma; 44 μg/ml in PBS pH 7.5) for 4 hours at room temperature and then excess biotin was removed by exhaustive dialysis against PBS. Wells were developed with avidin-conjugated horse-radish peroxidase (1:10,000; Dako, Ely, UK) and o-phenylenediamine (1mg/ml). The colour generated was measured in a plate reader (FLUOStar Galaxy, BMG Lab technologies, Offenburg, Germany). Quantitative data of the level of contamination on each band were obtained by comparison with standard curves generated using pooled, clarified stimulated human whole saliva (freshly collected from volunteer laboratory personnel) and pooled human serum (Sigma). Furthermore, purifed albumin and amylase were used as comparisons for the reactivity of the antibodies. Salivary amylase was purified from human parotid saliva by published methods[5]. Albumin was obtained from Sigma. Although albumin and amylase are present in both fluids, there are very marked differences in quantity. Data were extrapolated to determine the potential infective risk posed by the bands.

To determine the recovery of contaminating material from the bands after elution in PBS, each band was probed with the two antibodies to detect retained proteins. The resultant colour generated in solution was measured as above.

Statistical Methods

The sample size calculation was performed using data from Lowe et al[6]. They found that 34 percent of bands were contaminated following hand scrubbing, compared with 7 percent following ultrasonic cleaning. Using these data we estimated that we would require 30 patients to detect a similar difference at $p<0.05$ with a power of 0.90. An extra two patients were recruited to allow for potential loss of samples.

The proportions of Control (uncleaned) and Experimental (cleaned) bands demonstrating detectable levels of saliva were compared. The distributions of the data regarding volumes of detected fluid were examined and found to be positively skewed. The data from the Control band was paired with the data from the Experimental band on the contra lateral side of the arch for each participant. The Wilcoxon matched pairs signed rank test was used to test the hypothesis that there was no difference between the volumes of detected fluid on the Control compared with the Experimental band.

The proportions of bands with and without detectable bleeding were compared for those which demonstrated visible bleeding on removal of the band compared with those with no visible bleeding on removal. The volumes of detectable blood between bands with visible bleeding and the contra lateral band in the same or opposite arch with no visible bleeding were compared using the Wilcoxon matched pairs signed rank test.

The quantitative data from the antibody capture assay was used to assess the level of risk of cross-contamination from tried-in bands.

Results

The four bands randomly chosen from the selection tray, which had not been placed in the mouth, showed no detectable amylase or albumin. This demonstrates that contamination of the bands had not occurred prior to trying them in the mouth.

There was detectable amylase on 83 percent of bands that had not been subjected to ultrasonic cleaning. This compared with 33 percent of bands that had been through the ultrasonic cleaner. The median volume of saliva detected was 1.84μml (IQR 3.7) on uncleaned bands and 0μml (IQR 0.34) on cleaned bands (Figure 1). This was statistically significant (p=0.003).

There was detectable albumin on 59 percent of bands that had not been subjected to ultrasonic cleaning. This compared with 39 percent of bands that had been through the ultrasonic cleaner. Although the median volume of blood detected reduced from 0.03μml (IQR 0.07) on uncleaned bands to 0μml (IQR 0.04) on cleaned bands (Figure 2), this was not statistically significant (p=0.053).

Figure 3 shows the proportions of bands demonstrating negative and positive results for the detection of amylase and albumin before and after cleaning. The percentage of bands with no detectable contamination with either amylase or albumin rose from nine out of 64 (14%) before cleaning to 32 out of 64 (50%) of bands after cleaning. The number of bands with only amylase detectable fell from 17 (27%) before cleaning to 7 (11%) after cleaning. The number of bands with only albumin detectable rose from 2 (3%) before cleaning to 11 (17%) after cleaning and the number with both amylase and albumin detectable was 36 (56%) before cleaning to 14 (22%) after cleaning.

The presence or absence of visible bleeding was recorded in 124 out of the total 128 sites. In 18 out of 124 sites (14.5%) there was visible bleeding on removal of the band. There was

detectable albumin on 11 out of the 18 bands from these sites (61%). There was no significant difference in the volume of albumin detected on bands where bleeding was visible, compared with when bleeding was not visible on removal of the band ($p = 0.311$) (Figure 4).

Discussion

This study has found that 50 percent of molar bands, which have been tried for size in the mouth have detectable amylase, albumin or both, even after 15 minutes in an ultrasonic cleaning bath. The volume of detectable amylase was significantly reduced compared with uncleaned bands; however the reduction in the volume of albumin was not statistically significant.

Although the ELISA tests performed were not specific for blood or saliva, the concentration of amylase in saliva (46 ± 21 U/ml) is approximately 1000 fold higher than it is in blood (23-85 U/L) and the concentration of albumin in blood (34 – 54 mg/ml) is approximately 1000 fold higher than it is in saliva (39 ± 13 μg/ml)[7] . Therefore, we believe that blood is mostly responsible for the positive albumin results and saliva is mostly responsible for the positive amylase results. The volume of blood was estimated as the volume of serum with the amount of albumin detected by the test. It would, however be wrong to state categorically that everything with albumin is blood; therefore there is the possibility of some false positive results.

We are not certain why all bands that had not been through the ultrasonic cleaner did not show the presence of saliva in our assay. Pilot work showed that serum or saliva dried onto bands at room temperature for 30 minutes and then "extracted" into buffer showed greater than 90 percent recovery of the albumin or amylase. Samples, once taken, were immediately frozen and stored at -20°C until they could be assayed, which was up to several weeks. There is likely to have been some freeze drying during storage and so one possible explanation for failure to detect saliva on all bands is that drying may have led to precipitation of proteins/glycoproteins, which subsequently failed to completely resolubilise in the

extracting buffer. If this is the case then it seems reasonable to assume that our data are an underestimate of the level of contamination present, rather than an over estimate.

Effective cleaning of contaminated instruments prior to autoclaving is essential for successful sterilization. Whitworth et al[8] have shown that autoclaving alone failed to eliminate all test bacteria inoculated onto dental burs and the presence of blood and saliva increased the number of bacteria recovered. Moreover, other studies have found that ultrasonic cleaning does not fully remove accumulated debris from dental instruments. Aasim et al[9] showed that a significant minority of endodontic files (29%) still retained debris after 30 minutes ultrasonic cleaning and that there was no benefit in pre-soaking the files in an enzymatic cleaner. These authors concluded that the optimum time for cleaning was between 5 and 10 minutes in the ultrasonic cleaner, as longer exposures did not improve cleanliness.

Letters et al[10] examined 250 endodontic files collected from 25 general dental practices in Scotland. The files had been used to treat at least one patient and had been through the usual decontamination procedures for that particular practice, which in seven out of the 25 practices included ultrasonic cleaning. They found that 75 percent of the files showed evidence of residual contamination with debris and 6.8 percent had a positive Kastle-Meyer test for the presence of blood. Also, Smith et al [11] found that all of the 220 endodontic files collected from 22 dental practices had residual protein detected using a fluorescent assay based on reaction of proteins *o*-phthaldaldehyde/N-acetyl cysteine. The median quantity of protein was 5.4μg with a range from 0.5μg to 63.2μg.

The cleaning of endodontic files used to remove pulpal tissue and clean root canals or dental burs used to remove carious tooth tissue will be more of a challenge than a molar band tried on a tooth. Fulford et al[2] failed to grow any bacteria from tried-in bands that had been subjected to an enzymatic cleaner/disinfectant and then sterilized using either a downward displacement or a vacuum cycle autoclave and concluded that the potential risk of cross contamination between patients is minimal from properly decontaminated tried-in molar

bands. However this study has shown it is important for clinicians to remain vigilant with regard to cross infection control procedures. It made no difference to the volume of albumin detected from cleaned bands whether overt bleeding was observed or not after the band had been removed from the mouth. Therefore it is essential that all bands undergo adequate cross-contamination procedures prior to reuse.

The tests performed in this study are highly sensitive methods of detecting the presence of albumin. ELISA has an advantage over other tests, such as the Kastle-Meyer test, because it is not only possible to detect the presence or absence of albumin in small quantities, but it is also possible to quantify the amount. Therefore from these results it is possible to estimate the potential for cross infection by specific micro-organisms.

A serum level of Hepatitis B virus (HBV) DNA of 10^5 genome equivalents per ml (geq/ml) is considered sufficient for transmission of HBV to occur in a surgical setting[12]. The infective dose of HBV has been estimated at 20 - 1000 geq. The study by van der Eijka et al[12] also showed infected patients to have a median HBV DNA level in serum of 2.10×10^5 genome equivalents per ml (geq/ml), ranging from 373 to 4.13×10^9 geq/ml. By comparison the median HBV DNA level in saliva was much lower at 2.27×10^4 geq/ml and ranged from undetectable to 9.25×10^6 geq/ml.

The most contaminated band in this study showed an amount of albumin remaining after cleaning equivalent to 0.59μl blood. This amount of blood could indicate the presence of up to 2×10^6 geq HBV, around 2000 infective doses by injection, although this volume from most subjects would contain a maximum of only 1 infective dose. Using the interquartile data from our work it is likely that 0.04μl of remaining blood would contain 8.4 geq HBV, a level less than one infective dose. In the case of salivary contamination the risks are less. The maximum HBV level after cleaning is likely to be 30 - 300 infective doses with the majority being less than one dose. These figures do not take into account the effect of autoclaving, which would be expected to reduce the number of infectious HBV significantly, even if not all

virus particles are destroyed, although it is acknowledged that residual organic matter can shield a proportion of organisms present from the killing effect of heat and chemicals.

Taken together, therefore, the findings of this study reinforce the requirement for rigorous cleaning of orthodontic bands after try in and show that in rare cases there is a real risk of transmission of HBV between patients if cleaning is inadequate. It is essential, therefore, to find a consistently effective method of cleaning tried in molar bands. Washer-disinfectors have been found to be the most effective method for pre-sterilization cleaning of dental burs [8] and their use for the decontamination of tried in molars bands requires further investigation.

Conclusions

- Ultrasonic cleaning for 15 minutes reduces, but does not eliminate detectable salivary proteins (amylase) from tried-in bands.

- Ultrasonic cleaning for 15 minutes is less effective at removing detectable serum protein (albumin) from orthodontic bands.

- There is a need to investigate effective means of cleaning organic material from orthodontic materials if they are to be adequately sterilized and reused.

Acknowledgements

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Contributions

Philip Benson and Ian Douglas were jointly responsible for the study design, data analysis and interpretation; critical revision and final approval of the article. Philip Benson was responsible for patient recruitment, data collection and drafting of the article. Philip Benson is the guarantor.

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Figure 1

Boxplots showing the medians and interquartile ranges for the volume of saliva detected on the uncleaned bands (control) compared with cleaned bands (experimental).

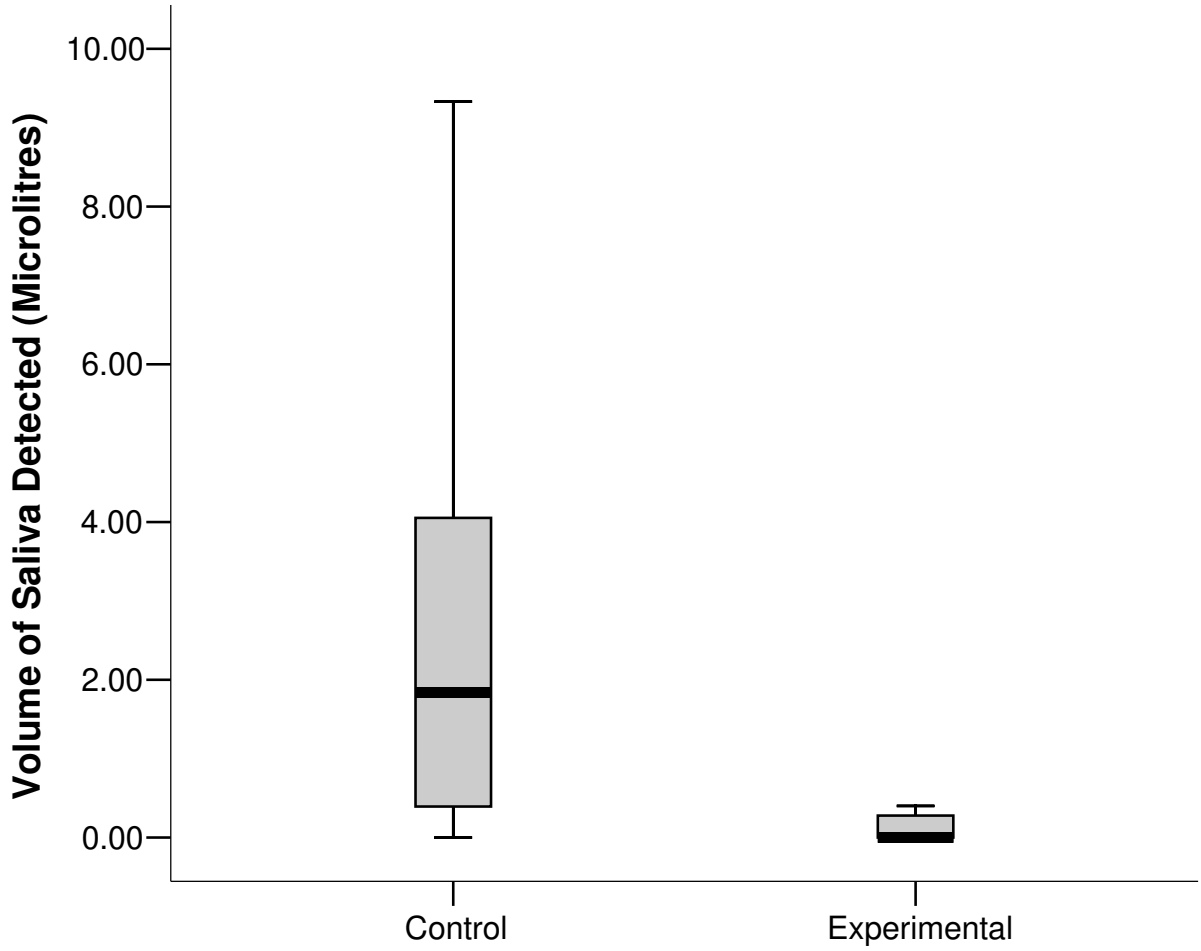

Figure 2

Boxplots showing the medians and interquartile ranges for the volume of blood detected on the uncleaned bands (control) compared with cleaned bands (experimental).

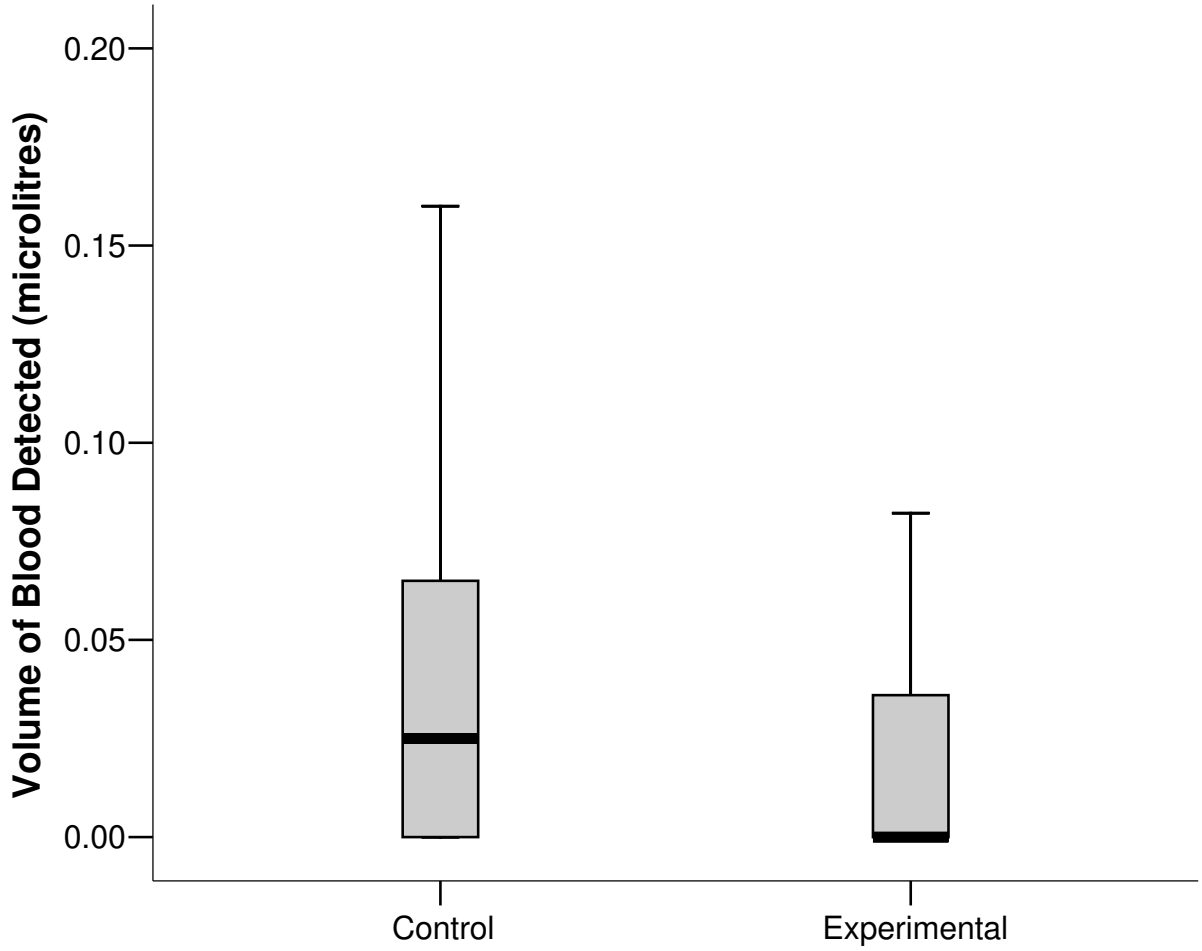

Figure 3

Graph showing the percentage of bands showing positive and negative detection of amylase and albumin before and after cleaning

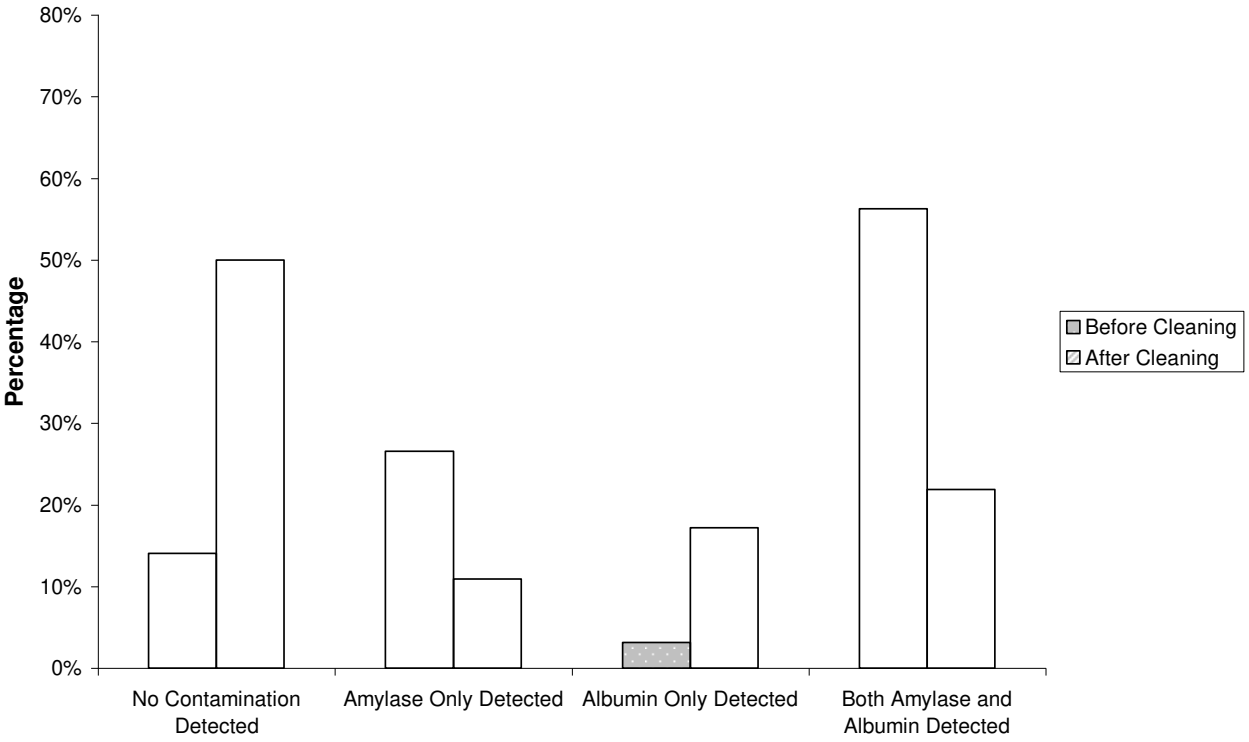

Figure 4

Boxplots showing the medians and interquartile ranges for the volume of blood detected on the bands where there was visible bleeding recorded following removal compared with bands where no visible bleeding was recorded following removal.