

# REDUCTION OF RESIDUAL PROTEIN ON RE-USABLE SURGICAL INSTRUMENTS USING PRE-TREATMENT FORMULATIONS

## Background

There is widespread concern about the potential for transfer of prion protein between surgical patients because of incomplete instrument decontamination and the resistance of prion to inactivation by steam sterilisation.

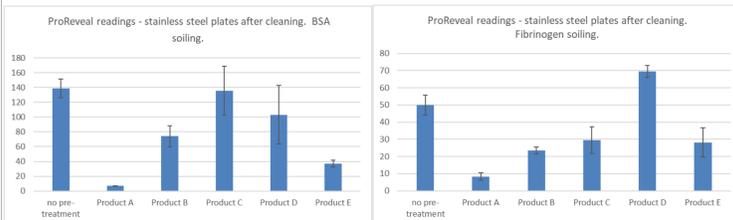
HTM 01-01 (UK Department of Health, 2016) states the objective that all surgical instruments should be decontaminated to a level where less than 5µg of residual protein is detectable per instrument side using a fluorescence based in-situ protein detection method. Implementation of these measures was due by July 2017 for neurosurgery and in all other surgical areas by July 2018. A key feature of this guidance is the observation that commonly used protein detection tests that rely on surface swabbing to remove residual protein for detection will not function correctly where protein is strongly adsorbed to surfaces, as is known to happen with prion proteins after drying.

The objective of this work was to measure the effectiveness of five commercially available pre-treatment materials in increasing protein removal from surgical instruments during reprocessing. These formulations are applied to soiled instruments as soon as possible after use, preventing soil drying on instrument surfaces before the cleaning process.

Since instruments, decontamination processes and associated equipment are all well-established in sterile services departments, our study sought to quantify the increase in protein removal achievable by inserting the use of a pre-treatment formulation into existing work-flows.

## Results

Protein removal from stainless steel plates using the different pre-treatment formulations is shown in charts 1 and 2 below. The results are shown in comparison with the amount of protein remaining on the test surface when no pre-treatment was applied. These results are reported as the numerical result from the ProReveal equipment in µg protein expressed as a mean of three repeats for each test.



Protein removal from haemostats is shown in charts 3 and 4 below in comparison with the result of washing only when no pre-treatment was used.

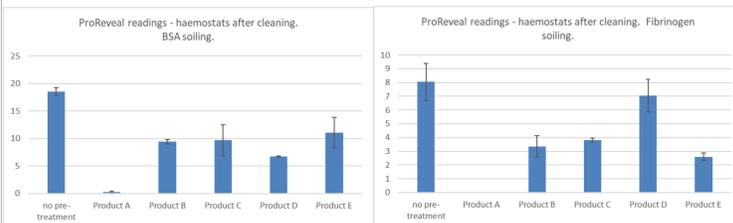


Chart 5 below shows a summary of data for all five products tested aggregated across all the substrate/protein combinations compared with no pre-treatment. These results are expressed as a percentage reduction in protein compared with the effect of washing only.

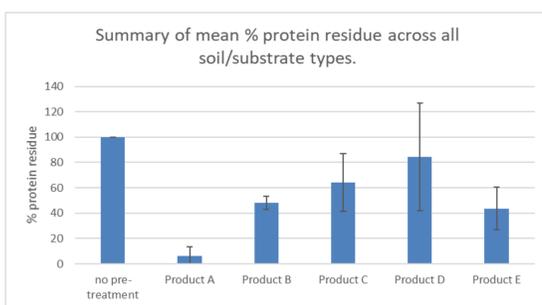
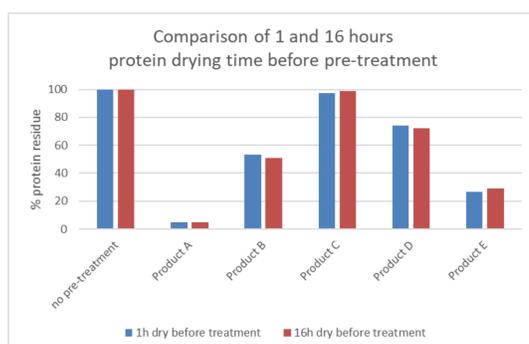


Chart 6 below gives a comparison of the protein residue levels measured after washing when BSA was allowed to dry on stainless steel plates for 1 and 16 hours at 30°C before pre-treatment formulations were applied.



## Experimental

### PROTEIN SOILING

Protein was applied to stainless steel plates and surgical instruments (haemostats). The test soils used were bovine serum albumen (BSA) and fibrinogen. The soils were applied to the test pieces as aqueous solutions of 200µg/ml. Various drying and cleaning processes were then used to simulate instrument use and reprocessing. The level of protein contamination and the drying method used were selected to ensure that significant quantities of proteins would remain on surfaces after processing.

### Preparation of test materials

Stainless steel plates (grade 316 150x100mm) and typical surgical haemostats were selected as the test substrates for this study. Test pieces were cleaned and degreased before use. Clean test pieces were evaluated using the ProReveal system to ensure freedom from any detectable protein residues to confirm the suitability of the preparation process.

Two protein soil solutions were prepared containing;

- 200µg/ml BSA (Fisher Scientific) in double distilled water
- 200µg/ml Fibrinogen (Sigma Aldrich) in double distilled water

Stainless steel plates were coated by pipetting 200µl of the appropriate protein solution onto the centre of the plate and spreading it evenly across the surface using a silicone blade before allowing the solution to dry for 3 minutes at room temperature. After initial drying, plates were placed in an incubator at 30°C.

Haemostats were part-immersed in the protein solution for 3 minutes, withdrawn and allowed to drain and air dry vertically for 3 minutes before being placed in an incubator at 30°C.

After the specified time in the 30°C incubator, the test materials were applied to the plates and instruments in accordance with the manufacturers' instructions for use.

### CLEANING PROCESS

Soiled test items were cleaned using a washer disinfectant (Getinge WD 14 Tablo). Washing conditions were adjusted to ensure a significant amount of residual protein remained on surfaces after washing so that the effect of the pre-treatment agents tested was emphasised.

Stainless steel plates were washed for three minutes at 55°C with 2ml/l of an enzymatic detergent (Getinge Clean Enzymatic detergent). The wash phase was followed by a two-minute water rinse and a drying cycle.

The protein soils used were found to be less adherent to the surfaces of haemostats, so these were subjected only to a two-minute water rinse process without detergent action in order to provide a useful level of protein residue to enable differentiation between the pre-treatment materials used.

### MEASUREMENT OF RESIDUAL PROTEIN

Residual surface protein levels after cleaning were assessed using the ProReveal system (Synoptics Health Ltd., Cambridge, UK.). This is based on a fluorescence reaction using an OPA based reagent to quantify residual protein in situ on surfaces and provide an image of the test item showing the location and magnitude of protein residues. At the time of writing, ProReveal is the only commercially available system meeting the criteria for protein detection laid down in the HTM 01-01 guidance. The system is claimed to have sensitivity to protein levels below 50ng, 1% of the maximum allowable residue per instrument side under HTM 01-01. Images produced by the system show residues of protein in yellow/orange. The degree of protein residue was assessed both by examination of the images and the numerical results generated by the ProReveal equipment.

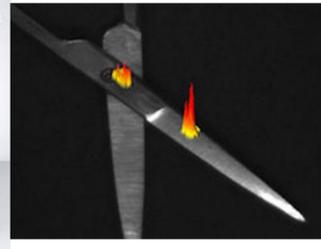


Fig.1. ProReveal equipment

Fig.2. Typical ProReveal image showing residual protein.

### EFFECT OF DRYING TIME ON PROTEIN REMOVAL

To assess the effect of prolonged drying time on protein removal, stainless steel plates were prepared as above by coating in BSA solution. The plates were allowed to dry at 30°C for 16 hours before being coated with the pre-treatment products. After one hour in contact with the pre-treatment at 30°C, washer disinfectant cleaning was carried out as detailed above.

## Method validation

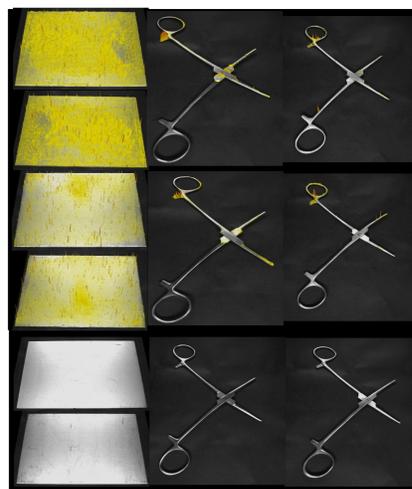
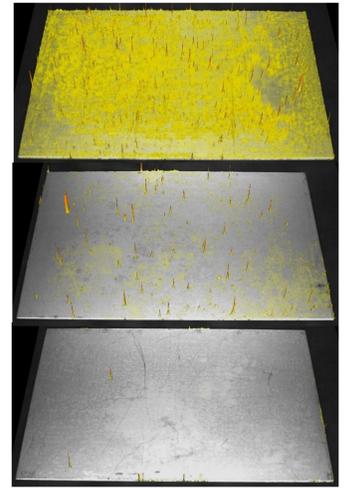


Fig. 3. (left) ProReveal images showing BSA soiling positive controls (top) Fibrinogen soiling positive controls (centre) and reagent only negative controls (lower).

Fig. 4. (right) ProReveal images showing wash process validation. Top, BSA loaded plate after drying. Centre, BSA loaded plate after drying and washing with no pre-treatment. Lower, BSA loaded plate after drying, pre-treatment application and washing.



Protein residue reduction was calculated as the difference between Pro Reveal numerical results for a washed test item without pre-treatment and the equivalent item with pre-treatment applied before washing.

## Discussion

It is clear from the results generated in this study that the use of a pre-treatment product before washing medical devices can significantly reduce levels of protein soil remaining on stainless steel surfaces. Significant variations in efficacy of protein removal performance between the pre-treatment formulations tested were noted. There were relatively small differences in the proportion of residual protein measured when comparing BSA with fibrinogen.

When BSA was allowed to dry on stainless steel plates for 16 hours at 30°C followed by a one hour treatment with the test products before cleaning, there was very little difference noted in the protein residue levels detected compared with 1 hour drying time. In all cases where the drying time was extended, there was a slight increase in the amount of protein remaining after cleaning but percentage removal was almost the same for both 1 and 16 hours drying. This is a useful result as it indicates that even if a pre-treatment product is applied with a limited contact time after prolonged protein drying, more than 90% reduction in residual protein can still be achieved compared with washing alone.

Future studies could include a comparison of pre-treatment materials with the additional use of plastic bags to retain moisture. Further study into the effects of protein drying time and drying conditions on removability with and without pre-treatment would also be useful.

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### Conflict of interest statement:

Product A in this study was formulated and developed by Quadralene Ltd, which owns the intellectual property for the product. The product is commercially available. The study was funded by Quadralene Ltd.