# Evaluation of the cleaning efficacy of Ruhof's Prepclean (Forever wet gel) for use as a moisture retaining formulation on surgical instruments.

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**Protocol: evaluation of moist gels (D021013)**
**Prepared For: Ruhof Corporation, USA**

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## Contents

# 1 Introduction

The study requested was to perform an evaluation of Prepclean as a moist retaining gel formulation. Experiments were conducted to test the gel formulation as a moist retaining gel, ability to dry and polymerise along with checking its compatibility with both alkaline and enzymatic detergents. The product was also tested on surgical instruments in a hospital SSD setting to evaluate the benefits of keeping instruments moist using Prepclean compared to the dry approach. Animal Brain tissue and homogenate was used in the study as it has been demonstrated in our laboratory that brain protein strongly adheres to stainless steel and would provide a more realistic test of the cleaning efficacy of wash detergents.
Analysis using the novel fluorescent protein detection technology was performed in a controlled laboratory where the method is optimised and validated.

# 2 Materials

- **Rat brains**: Rat brains were collected from male Wistar rats (Charles river).
- **Soil**: 10% w/v Pig Brain Homogenate (Prepared in the research labs at Bart's)
- **SS tags**: 316 grade stainless steel tags cut and polished (Surgical holdings UK)
- **Moist retaining gels**: Ruhof Prepclean (Forever wet).
- **OPA/NAC reagent**: Patented reagent prepared in the lab
- **Protein Detection System**: Patented prototype made in Cambridge by Syngene ltd.
- **Wash detergents**:
  - Enzymatic cleaner (Ruhof)
  - Alkaline detergent (Leading UK brand)

# 3 Product

Prepclean (batch number: 345PFW32) was provided by Ruhof corporation, USA. The product is described as Prepclean Forever wet and claims the following on its label:

1. Keeps soiled instruments moist
2. Eliminates dried on Bio-burden
3. No more messy spills
4. Stops splashing during transportation.

# 4 Gel Characteristics

The gel is transparent and viscous without any odour. When sprayed on stainless steel tags and instruments it adheres to the steel and runs down the sides slowly covering any blood and tissue on the surface (Figure 1).

**Figure 1**: **An image of the gel when sprayed on stainless steel surface**

## 4.1 Gel weight when bagged

Tests were carried out to calculate the % loss of applied gel weight on tags. The SS tags were each individually weighed and the weight of the applied gel noted. The tags were bagged appropriately for 3, 6, 24, 48, 72 and 100 hours and reweighed. The difference in weight is calculated as % loss in applied weight and plotted as a graph (Figure 2).

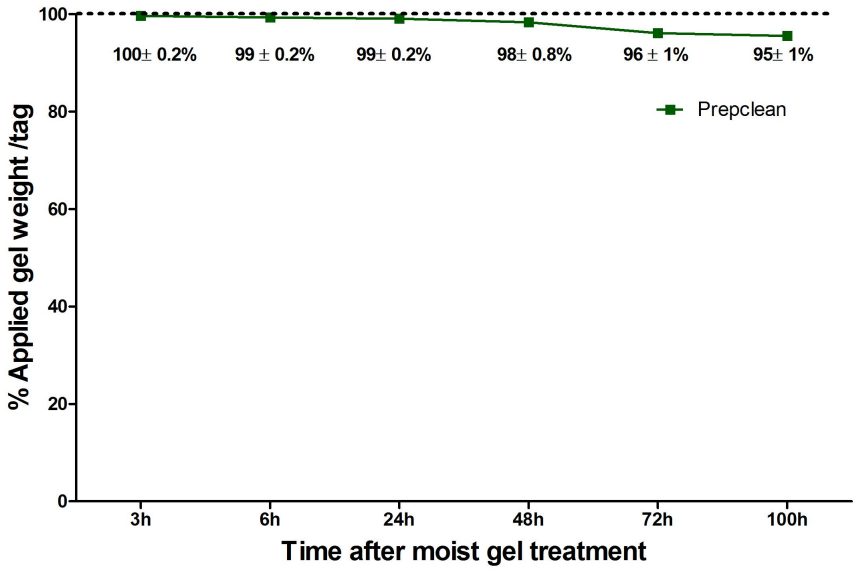


**Figure 2: A measurement of the moisture retaining ability of the gel when bagged with samples**

When the tags containing the gel were bagged appropriately, the gel was found to be maintaining its gel characteristics even after 100h (Figures 3 A and B)


**Figure 3**: **A SS tag sprayed with Prepclean and left bagged for 100 h. the gel retained its moist characteristics.**

## 4.2 Gel weight when left open

Tests were carried out to calculate the % loss of applied gel weight on tags when the tags are not bagged. The SS tags were each individually weighed and the weight of the applied gel noted. The tags were then left to dry at room temperature exposed to air for 3, 6, 24, 48, 72 and 100 hours and reweighed. The difference in weight is calculated as % loss in applied weight and plotted as a graph (Figure 4).

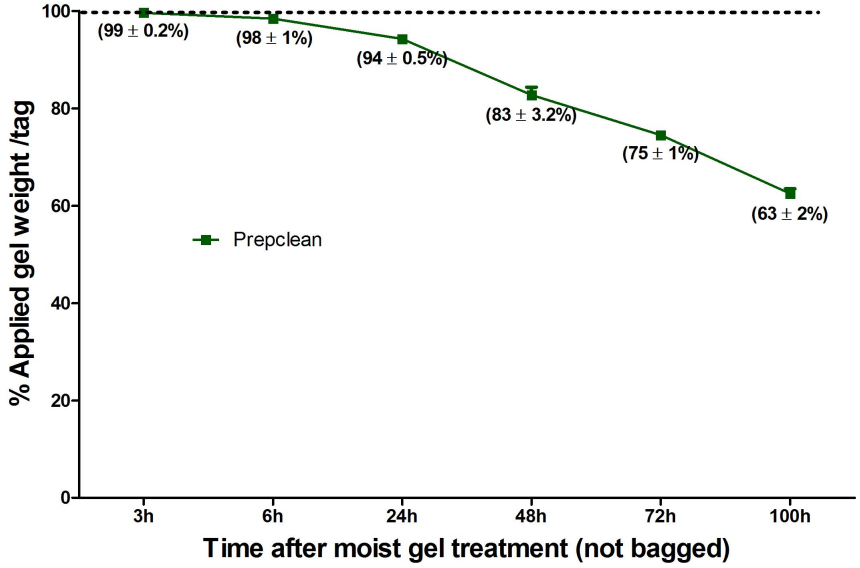


**Figure 4**: **A measurement of the moisture retaining ability of the gel when not bagged with samples (Open air dried).**

After 72 h the gel loses some of its characteristics retracts from areas where it has been sparse to begin with. This sometimes includes areas where protein has been contaminated. (Figure 5).

**Figure 5**: **Prepclean dried on stainless steel tag for 72 h**

# 5 Prevention of protein adhesion over time

SS tags were washed in an AWD and verified with the OPA/NAC reagent that they are clean. The tags were individually engraved as A (1 – 24) and B(1 – 24) and contaminated with protein (PBH). The contaminated tags and the controls were treated with Prepclean and bagged as shown in the table below for various time points in reverse order. This allows for all the time points being washed at the same time in the same washer for comparable results. Tags A (1 – 24) were used for testing drying of protein on surgical steel without any gels. Tags B (1 – 24) were used to test the prevention of drying of brain protein on surgical steel with the use of Prepclean.
All tags were contaminated with protein (4 spots ($100\mu l$) of PBH ($1mg/mL$) each containing $100\mu g$ of total brain proteins) apart from the negative controls.

| | |
|---|---|
| A) 3h protein tags | 1 - 4 |
| B) 6h protein tags | 5 – 8 |
| C) 24h protein tags | 9 – 12 |
| D) 48h protein tags | 13 – 16 |
| E) 72h protein tags | 17 – 20 |
| F) 100h protein tags | 21 – 24 |

The washed and sterilised tags were analysed for

1. residual protein (*ProReveal technology*)
2. brown spot formation: virtual observation of tags with naked eye and photographs (scoring 0-5)
3. visible corrosive effects or pitting on the steel surface: virtual observation of tags with naked eye and photographs (scoring 0-5).

# 6 Results

Protein contaminated tags that were not treated with Prepclean had significantly more residual protein compared to the corresponding time points with Prepclean treatment. Dry control tags had $8 \pm 3\%$ residual protein at 3h compared to no protein found on the tags treated with Prepclean. The % residual protein values were 0,0,0,0.3 $\pm$0.5, 0.8$\pm$ 1, and 1 $\pm$ 1 for 3, 6, 24, 48, 72 and 100 h respectively when Prepclean was sprayed on the tags after 3h of contamination. When the protein was left to dry the % residual protein values were 8$\pm$ 3, 11$\pm$ 13, 28$\pm$10, 53$\pm$7, 57$\pm$9, and 63$\pm$8 at 3, 6, 24, 48, 72 and 100 h respectively (Figure 6). The typical fluorescent image of the tags is shown in Figure 7. The results show that Prepclean significantly prevents the amount of residual protein by preventing the adhesion of protein to stainless steel. Even after 100 h in a bagged environment there is just about 1 % remaining on the tag with $400\mu g$ protein to begin with. The gel did not polymerise on the steel surface and was easily washed by both the alkaline and enzymatic washes tested here.
There was no visible discolouration on the tag surface.

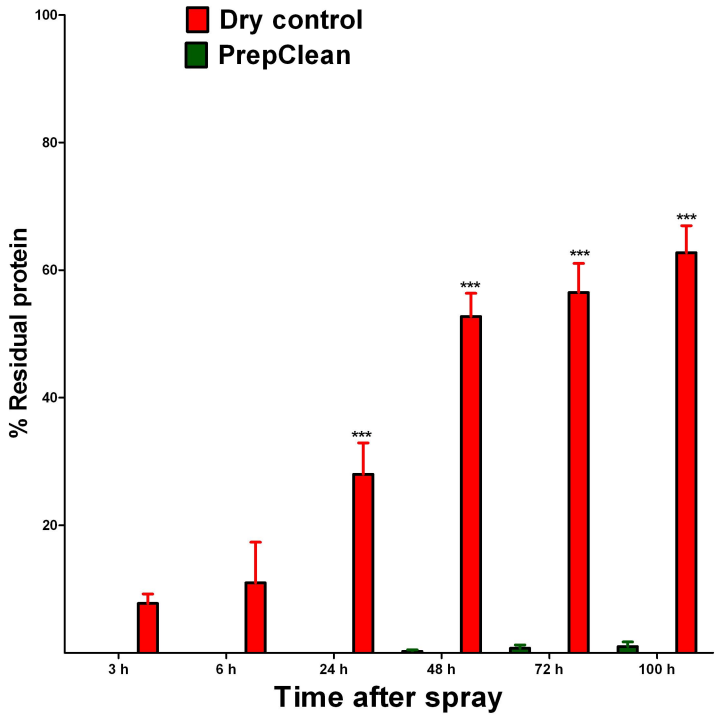


**Figure 6**: **Prevention of protein adhesion by Prepclean. Tags with protein were dried for 3 h and sprayed with prep clean for various time points. The respective dry controls were bagged without Prepclean treatment.**

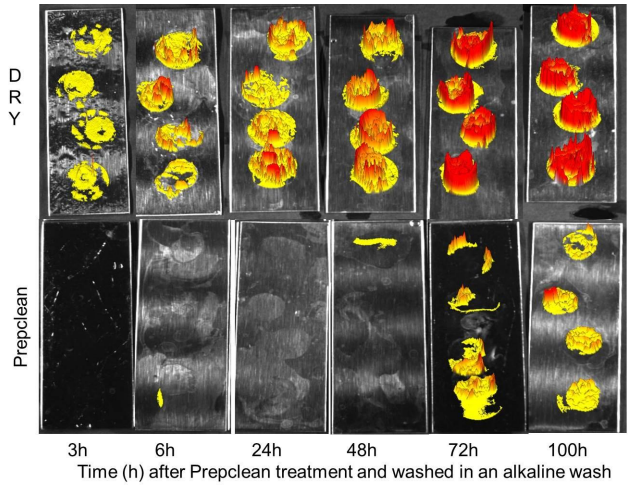


**Figure 7**: **Alkaline wash**

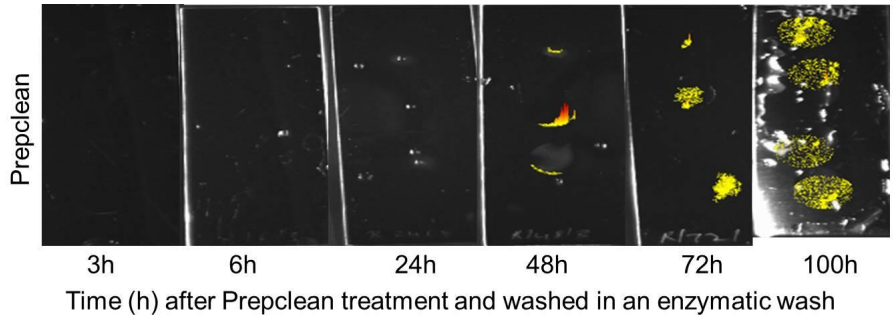


**Figure 8**: **Enzymatic wash**

# 7 Aiding removal of dried protein

SS tags were washed in an AWD and verified with the OPA/NAC reagent that they are clean. The tags were individually engraved as C (1 – 24) and D (1 – 24) and contaminated with protein (PBH). The contaminated tags and the controls were bagged as shown in the table below for various time points in reverse order without any moist gel treatments. Tags C (1 – 24) were not sprayed with any moist gels and tags D (1 – 24) were treated with Prepclean for 3 h after the experimental drying times before an alkaline or an enzymatic wash.

| | |
|---|---|
| A) 3h protein tags | 1 - 4 |
| B) 6h protein tags | 5 – 8 |
| C) 24h protein tags | 9 – 12 |
| D) 48h protein tags | 13 – 16 |
| E) 72h protein tags | 17 – 20 |
| F) 100h protein tags | 21 – 24 |

The washed and sterilized tags were analyzed for residual protein(*ProReveal Technology*)

# 8 Results

Contaminated stainless steel tags with protein dried for up to 100 h had significantly less residual protein compared to their respective dry controls without moist gel treatments when washed with an enzymatic or an alkaline detergent. The dry controls in an enzymatic wash had $0.5 \pm 0.2$, $3 \pm 1$, $19 \pm 4$, $33 \pm 4$, $35 \pm 10$ and $50 \pm 4$ respectively for protein dried for 3, 6,24,48,72 and 100h respectively. The corresponding moist tags treated with Prepclean gel had residual protein only on tags dried for 72 and 100 h with $1.3 \pm 0.5$, and $1 \pm 0.7$ % respectively (Figure 9). A typical fluorescent image shows some residual protein at 72 and 100 h (Figure 10).
In an alkaline wash, the dry controls had $9 \pm 1.5$, $13 \pm 7$, $31 \pm 5$, $66 \pm 6$, $64 \pm 8$ and $70 \pm 5$ % residual dried proteins for tags dried for 3, 6, 24, 48, 72 and 100 h respectively. The corresponding Prepclean treated tags had $2 \pm 1$, $14 \pm 3$, $18 \pm 1$ and $19 \pm 3$ % residual protein for 24, 48, 72 and 100 h respectively. The 3 and 6 h tags had no residual protein remaining when treated with Prepclean under these wash conditions (Figure 11). A typical fluorescent image of residual protein is shown in Figure 12.

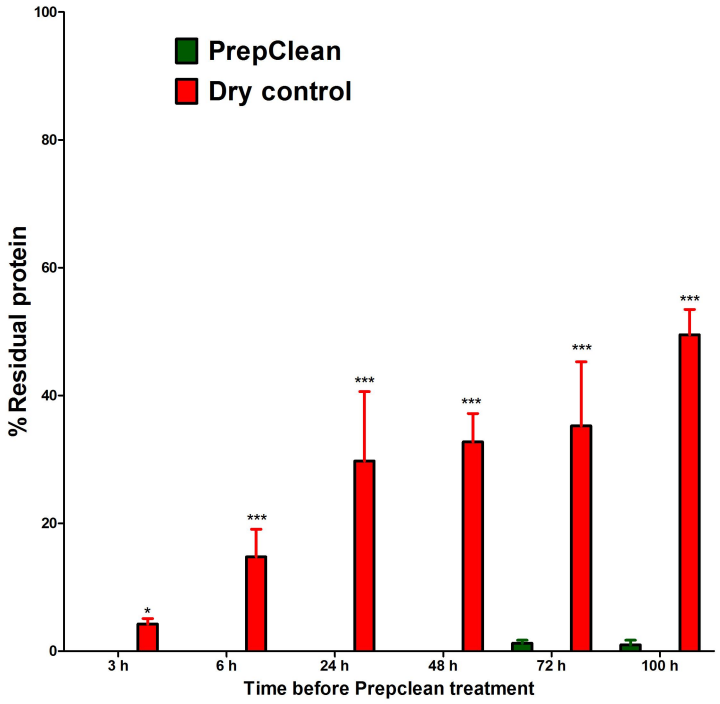


**Figure 9**: **Removal of dried protein by Prepclean**

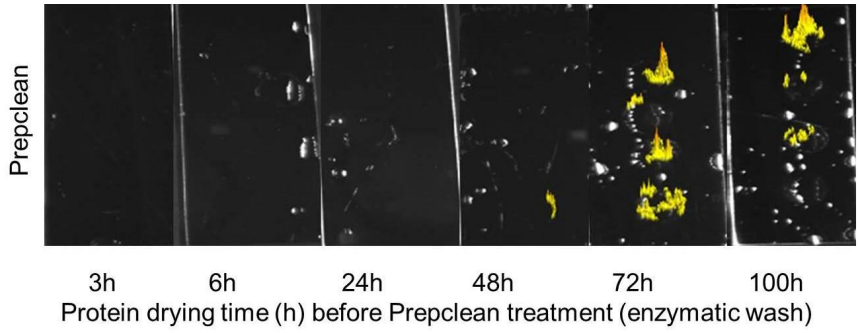


**Figure 10**: **A typical image of tags washed in an enzymatic detergent**

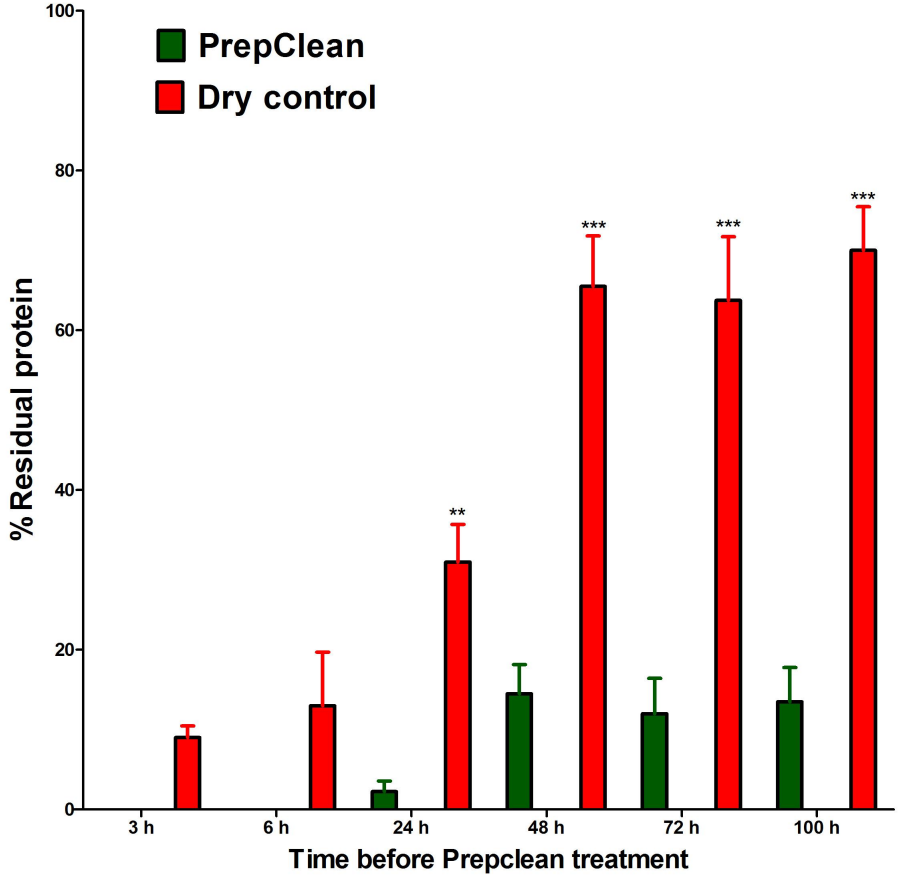


**Figure 11**: **Removal of dried protein by Prepclean when washed in an alkaline detergent**

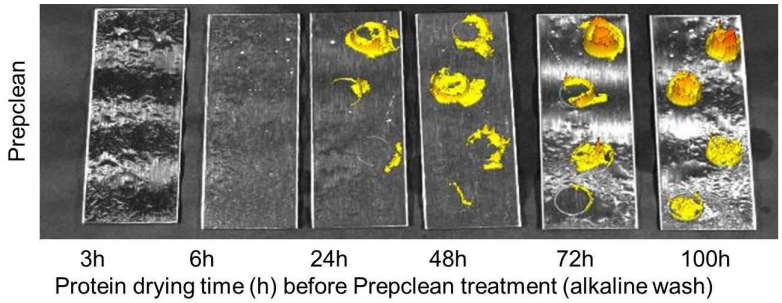


**Figure 12**: **A typical image of tags washed in an alkaline detergent**

# 9 Compatibility with alkaline detergents

SS tags contaminated with PBH and control uncontaminated tags were treated with Prepclean. The tags were bagged air tight for 3h and 72h before being washed in a validated AWD using an alkaline wash. After the wash, the tags were inspected for any staining or residues on the steel surface. All the tags were clean and had no residual gel matrix left on the steel surface

# 10 Compatibility with enzymatic detergents

SS tags contaminated with PBH and control uncontaminated tags were treated with Prepclean. The tags were bagged air tight for 3h and 72h before being washed in a validated AWD using an enzymatic wash. After the wash, the tags were inspected for any staining or residues on the steel surface. All the tags were clean and had no residual gel matrix left on the steel surface

# 11 Test of Prepclean on surgical instruments in a hospital

12 reusable neuro-surgical instruments were used for the study. The instruments were initially washed in an SSD to remove any potential protein contamination due to handling. The washed instruments were packed into sterile bags in the controlled clean room environment of an SSD before transported to the laboratory.
The instruments were contaminated with rat brain tissue by manipulating whole brains. The weight of adhered tissue was determined using a 4-place analytical balance. The brains were then homogenised and the total protein

measured using a Biuret reagent (Bio-RAd Ltd). Following the manipulations the instruments were allowed to dry at room temperature for 3 h before being treated with Prepclean moist gel. The moist treated instruments and the dry controls were bagged in clear plastic bags and left to dry for 48 h. after the stipulated drying time the instruments were transported to the SSD where they were using standard cycles.
The washed and dried instruments were sealed in sterile bags in the clean SSD environment and returned to the laboratory at Bart's for analysis. The residual protein on each instrument was measured against a protein (BSA) standard using the OPA/NAC protein detection method.

## 11.1 Results

A BSA standard was measured before the analysis of the surgical instruments washed in the SSD. The linear regression of the fluorescence is shown in Figure 13.

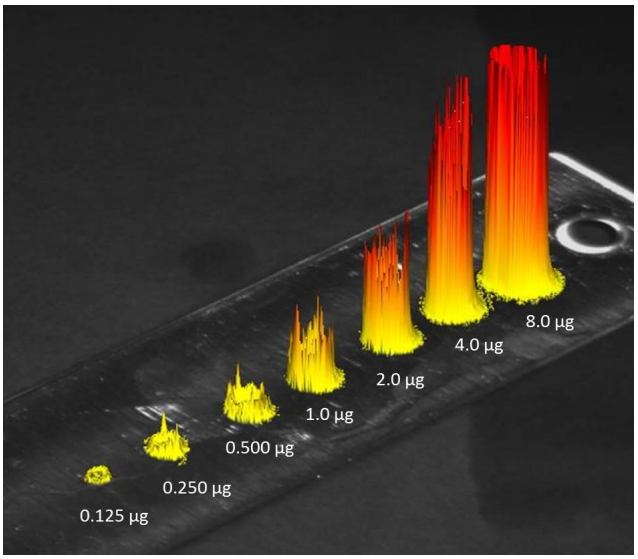


**Figure 13**: **A fluorescent image of the BSA standard** ($0$–$8\mu g$) **using the OPA/NAC protein detection method. The** $R^2 = 0.99$**. Equation of the line is** $Y = 4E + 06x - 559010$**.**

LoD: The limit of detection of the method is calculated from a sub microgram standard.
The LoD of the method is 5 ng.
LoQ:The LoQ of the method is 17 ng.
Six different instruments were tested for each condition. 12 instruments in total were divided as groups A and B. The total protein content of the adhered tissue was $270 \pm 74$ and $298 \pm 19 \mu g$/instrument for groups A and B respectively. Instruments in Group A were used as dry controls and were bagged for 48 h after the initial 3h open air drying. Group B was left to dry for 3 h and sprayed with Prepclean gel so that the instruments were sufficiently covered in all areas of contamination that can be visibly seen. The bags were then bagged and left for 48h. After an alkaline wash, the percentage protein remaining per instrument was calculated by quantifying the total protein per side of the instrument and correcting for both sides (Tables 1 and 2). The average protein remaining on the dry controls was $18 \pm 13 \mu g$ total protein (n = 6). The Prepclean treated moist retained instruments had $0.2 \pm 0.2 \mu g$ total protein (n = 6). Three of the six instruments analysed had no detectable protein on the instruments using the current sensitive technique (Figures 14, 15). The results indicate significant improvement ($P < 0.001$) in instrument cleanliness using Prepclean when compared to the dry approach (Figure 16).

**TABLE 1**

**A summary table of instruments washed with alkaline detergent when kept dry.**

| INSTRUMENT | Total Protein in the adhered tissue ($\mu g/instrument$) | Protein remaining after washing ($\mu g/instrument$) | % Protein remaining/ Instrument |
|---|---|---|---|
| AD1 | 234 | 6.4 | 2.7 |
| AD2 | 143 | 12.8 | 9.0 |
| AD3 | 356 | 19.2 | 5.4 |
| AD4 | 302 | 37.2 | 12.3 |
| AD5 | 302 | 26.4 | 8.7 |
| AD6 | 284 | 4.01 | 1.4 |
| mean | 270.2 | 17.7 | 6.6 |
| stdv | 73.6 | 12.6 | 4.2 |

**TABLE 2**

**A summary table of instruments washed with alkaline detergent when kept moist using Prepclean.**

| INSTRUMENT | Total Protein in the adhered tissue ($\mu g/instrument$) | Protein remaining after washing ($\mu g/instrument$) | % Protein remaining/ Instrument |
|---|---|---|---|
| BM1 | 265 | 0 | 0.0 |
| BM2 | 302 | 0 | 0.0 |
| BM3 | 294 | 0.6 | 0.2 |
| BM4 | 296 | 0.3 | 0.1 |
| BM5 | 321 | 0.1 | 0.0 |
| BM6 | 312 | 0 | 0.0 |
| | 298.3 | 0.2 | 0.1 |
| | 19.23 | 0.24 | 0.08 |

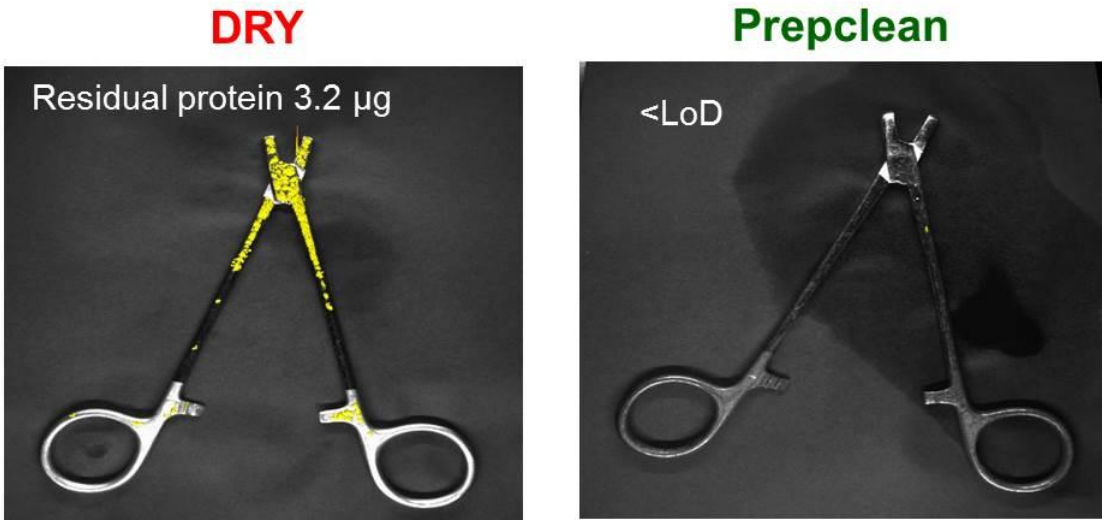


**Figure 14**: **Typical fluorescent images of contaminated needle forceps washed in an SSD using Alkaline detergent under dry and moist conditions. The pseudo colour from yellow to red depicts more protein in the red region.**

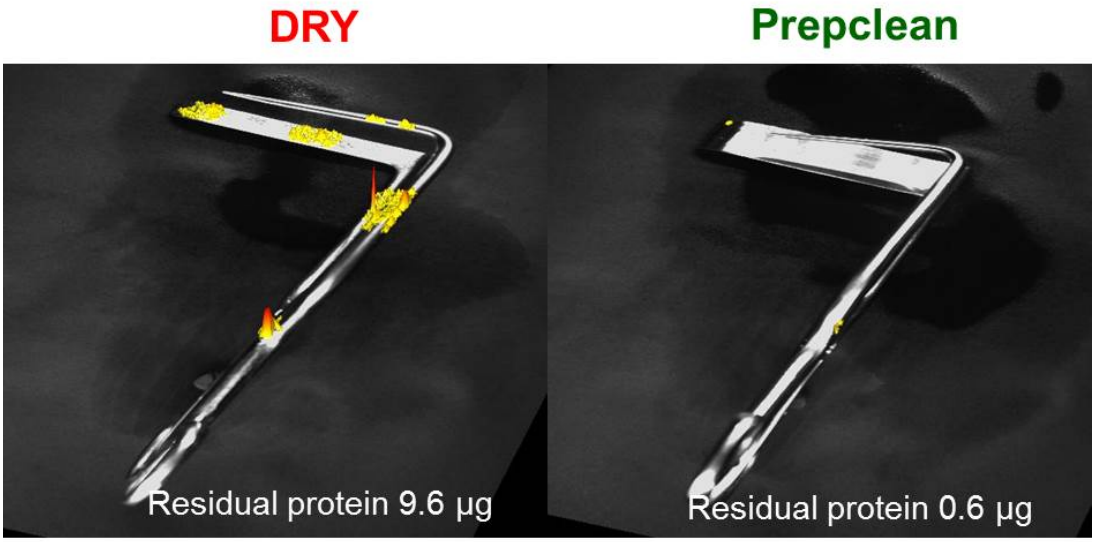


**Figure 15**: **Typical fluorescent images of contaminated instruments washed in an SSD using Alkaline detergent under dry and moist conditions. The pseudo colour from yellow to red depicts more protein in the red region.**

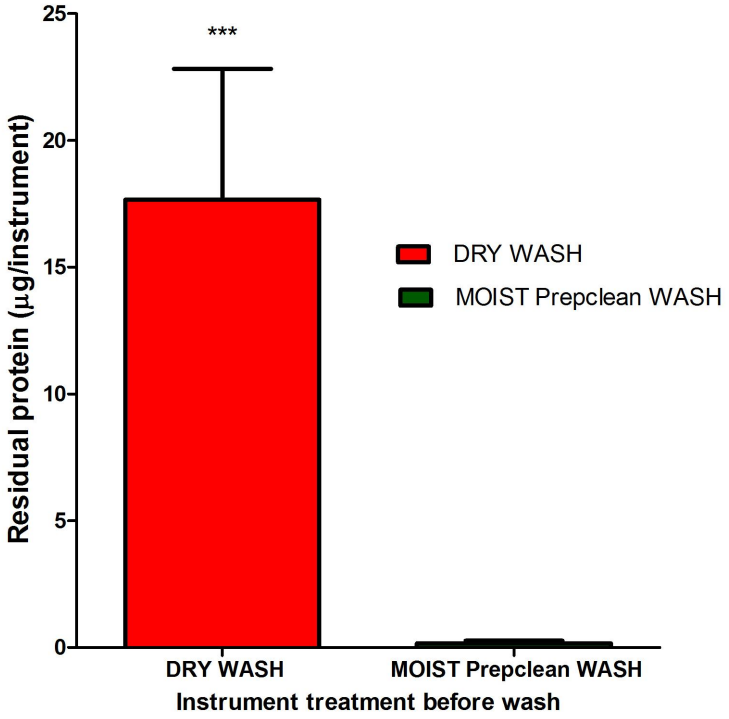


**Figure 16**: **Typical fluorescent images of contaminated needle forceps washed in an SSD using Alkaline detergent under dry and moist conditions. Instruments used to manipulate rat brain tissue for 5 minutes following which the instruments were air dried for 3 h before treating them with Prepclean for moist conditions for 48 h. The weight of the adhered tissue was measured and the total protein calculated. The instruments were washed in a hospital SSd using an alkaline detergent. The washed instruments were sprayed with the OPA/NAC reagent and the residual protein was measured against a BSA std. Results are mean ± SEM. N=6 both groups *** = $P < 0.001$. Statistical difference was determined by ANOVA.**

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# 12 Conclusions

- The gel adheres to stainless steel on contact and is viscous enough to cover contaminated blood and tissue.
- The gel stays moist and does not dry over 72 h when bagged air tight.
- The Prepclean gel prevents protein adhesion and drying on stainless steel up to 72h when washed with the test enzymatic cleaner and upto 48h when washed with the test alkaline detergent.
- The Prepclean gel significantly aids removal of dried protein even after 48 h when washed with the test enzymatic cleaner and after 24h when washed with a typical alkaline detergent.
- The test concludes that Prepclean does not leave any residue either by itself or in combination with an ezymatic or an alkaline detergent.
- The Prepclean gel is compatible with both enzymatic and alkaline detergents.
- No aerosols are generated while spraying the gel.

# 13 Declaration

This study was conducted using the agreed protocol D021013 A and the validated and approved protein detection measurement method developed in our laboratory. This report accurately describes the procedures used and the results and conclusions accurately reflect the raw data from the study. The original records of this report are stored in Deconsure lab records.
This report is for the exclusive use of Ruhof Corporation, USA. These results are valid and relate only to the instruments and samples (batch numbers) used and tested here. Any extrapolations must be confirmed by the scientist and a written communication is required. The significance of the data is subject to the wash detergents used in the SSDs and the Batch numbers thereof. Any liability for the loss or damage resulting from the publication or use of these results in company literature is solely upon the sponsor of study and not that of the scientist that performed the tests.

Dr N K Nayuni
Scientist
Deconsure Ltd
Signed 25/11/2013