Evaluation of the cleaning efficacies of Endozime Xtreme Power and an Alkaline Detergent.

Protocol: Quantitative surgical instrument study in an SSD (V020712).

Prepared For: Ruhof Corporation, USA

Prepared by:

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Introduction:

The cleaning efficacy of two wash detergents were performed using the protocol for wash detergents V020712 already agreed with Ruhof. The study requested was to perform a quantitative surgical instrument study in an SSD. Rat brain tissue was used in the study as it has been demonstrated in our laboratory that proteins in brain tissue strongly adheres to stainless steel and provides a realistic test of the cleaning efficacy of wash detergents.

Single use stainless steel instruments were used for the study. Analysis used the novel fluorescent protein detection technology developed at Barts where the method is optimised and validated.

Materials

Rat brains: Rat brains were collected from male Wistar rats (Charles River Labs).

OPA/NAC reagent: Patented reagent prepared in the lab

ProReveal System: Patented protein detection System. .

Surgical Instruments: Purchased from Surgical Holdings Ltd (U.K).

Washer: BHT Hygienetechnik

Wash detergents:

1) Endozime XP (Ruhof)
2) Major Alkaline brand

Wash cycle

Programme: See appendix A for full details.

Cleaning time in the presence of Endozime XP = 10 minutes 50 seconds.

Temperature: 45°C – 55°C

Dilution of detergent

1) Endozime XP : 2ml/L in RO water
2) Alkaline: 4ml/L in RO water

Method:

Quantitative surgical instrument study (In an SSD wash using BHT Hygienetechnik washer disinfector)

40 new single use surgical instruments purchased for the study. The instruments were initially washed in an SSD to remove any manufacturing debris and potential protein contamination due to handling.

The washed instruments were packed into sterile bags in the controlled clean room environment of an SSD before transport 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 48 h.

The instruments were immediately transported to the SSD where they were cleaned in one of two hospital washer disinfectors (**BHT Hygienetechnik)** using standard cycles. Each AWD had been optimised for one of the wash detergents by engineers using tests according to the manufacturers' guidelines.

The washed and dried instruments were sealed in sterile bags in the clean SSD environment and returned to the laboratory at Barts for analysis.

The residual protein on each instrument was measured against a protein (BSA) standard using the OPA/NAC protein detection method. (see appendix B)

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 1.

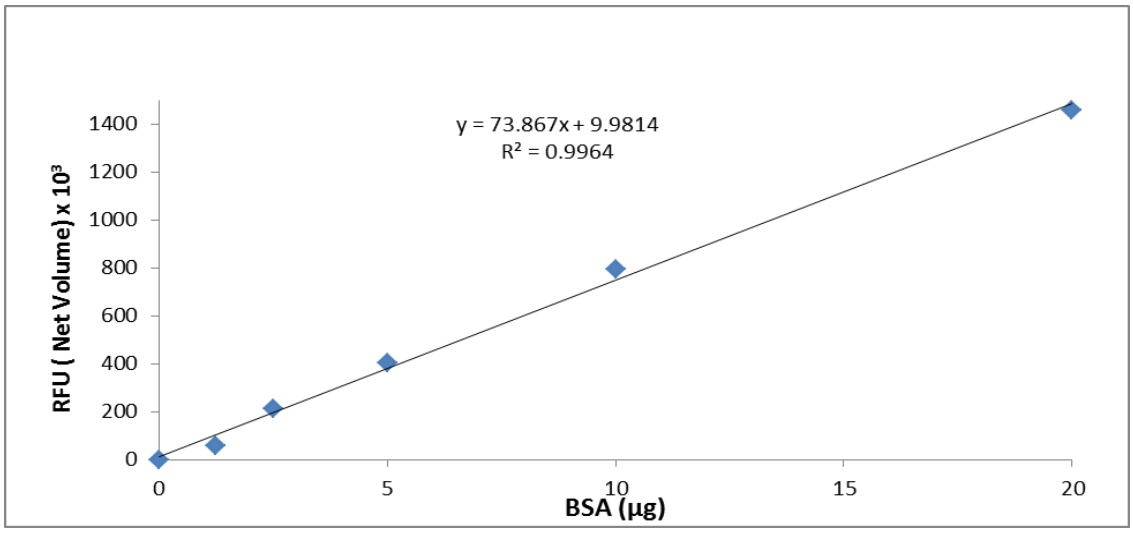


Figure 1: *Linear regression of BSA (0 – 20 µg) using the OPA/NAC protein detection method. The RFU is plotted against the protein concentration. The* R^2 *= 0.99. Equation of the line is Y=73.9x + 9.98.*

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 types (S,C,B,E,D and P See Appendix B for details) of instruments were tested under each condition. The total protein content of the adhered tissue was 369 ± 48 and 336 ± 28 µg / instrument on forceps (Type E) and scissors (Type D) respectively (Tables 2 and 3). The percentage protein remaining per instrument after an Alkaline wash in an SSD under the described conditions was 7.7 ± 1.6 (n = 6) and 13.9 ± 0.9 (n = 8) for forceps (Type E) and Scissors (Type D) respectively (Figure 2). When the instruments were washed with Endozime XP in an SSD the % Protein remaining per instrument was 0.3 ± 0.2 (n = 6) and 2.3 ± 0.9 (n = 8) for forceps (Type E) and Scissors (Type D) respectively (Figure 2). The % protein remaining per instrument on both the

instrument types was significantly less (P<0.001) when washed with Endozime XP compared to an Alkaline wash using a BHT Hyginetechnik AWD (Figure 2, 3,4 and 5).

Alkaline Wash				
INSTRUMENT	**Total Protein in the adhered tissue (µg/Instrument)**	**RFU (Net volume)**	**Protein remaining after washing (µg/Instrument)**	**% Protein remaining/ Instrument**
MPS1	666.	7637068	103.25	15.48
MPC1	443	682314	9.10	2.05
MPB2	116	59638	0.67	0.58
MPB1	245	65324	0.75	0.30
MPE2	121	600595	8.00	6.60
MPE1	235	1365267	18.35	7.78
MPE4	84	487314	6.46	7.64
MPE3	461	4585914	61.95	13.41
MPE5	396	367251	4.84	1.22
MPE6	485	3517194	47.48	9.78
UD11(P)	388	4759824	64.30	16.53
UD32(P)	265	2786146	37.58	14.13
UD17(P)	270	3149155	42.50	15.74
UD33(p)	562	5410828	73.12	13.01
UD15(P)	582	6262106	84.64	14.53
UD16(P)	227	2495100	33.64	14.78
UD12(P)	233	2508778	33.83	14.47
UD37 (P)	239	1470835	19.78	8.25
MPA1	616	6260796	84.62	13.72
MPP1	62	104056	1.27	2.04

Table 1: A summary table of instruments washed with Alkaline detergent in the final analysis.

Endozime XP Wash				
INSTRUMENT	**Total Protein in the adhered tissue (µg/Instrument)**	**RFU (Net volume)**	**Protein remaining after washing (µg/Instrument)**	**% Protein remaining/ Instrument**
MES1	409.0	705043.	9.41	2.30
MEC1	511.8	775564	10.36	2.03
MEB2	294.2	<LoD	<LoD	<LoD
MEB1	205.5	<LoD	<LoD	<LoD
MEE4	296.2	<LoD	<LoD	<LoD
MEE2	304.3	<LoD	<LoD	<LoD
MEE6	467.5	124296	1.55	0.33
MEE1	483.6	31316	0.29	0.06
MEE3	439.3	35960	0.35	0.08
MEE5	652.5	760100	10.16	1.55
UD13 (E)	350.6	390538	5.15	1.47
UD14 (E)	298.2	398933	5.27	1.77
UD28 (E)	255.9	939585	12.58	4.92
UD27 (E)	328.4	1900277	25.59	7.79
UD21 (E)	300.2	240643	3.12	1.04
UD19 (E)	253.9	177431	2.27	0.89
UD 18 (E)	396.9	59193	0.67	0.17
UD38 (E)	429.2	60548	0.68	0.16
MEA1	346.6	<LoD	<LoD	<LoD
MEP1	98.7	<LoD	<LoD	<LoD

Table 2: A summary table of instruments washed with Endozime XP in the final analysis.

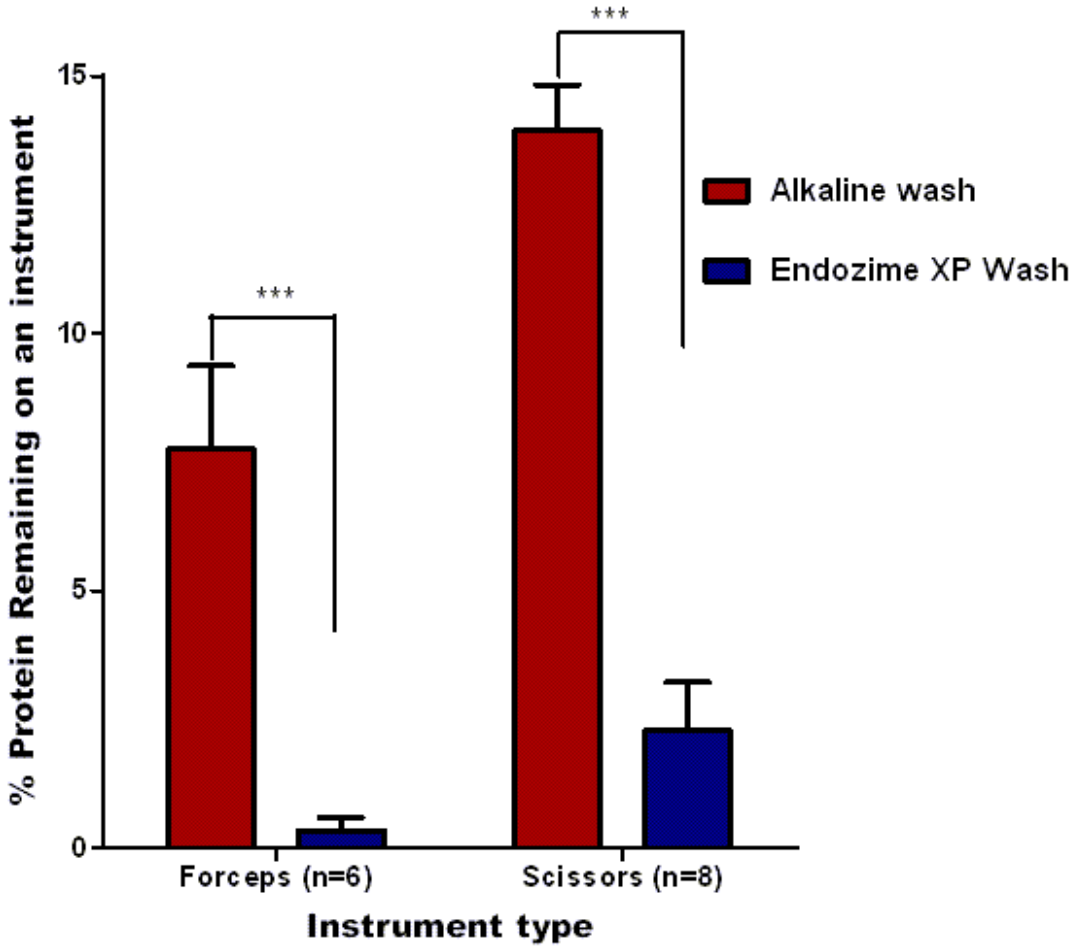


Figure 2: Comparative removal of dried on brain proteins using AWD in an SSD.

*Forceps and Scissors were used to manipulate rat brain tissue for 5 minutes following which the instruments were air dried for 48 h. The weight of the adhered tissue was measured and the total protein calculated. The instruments were washed in a BHT hyginetechnik AWD in an SSD using the cycles optimised for the Alkaline (Red bars) and Endozime XP (Blue bars). 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 for forceps and n=8 for scissors in each wash. *** = P<0.001. Statistical difference was determined by ANOVA.*

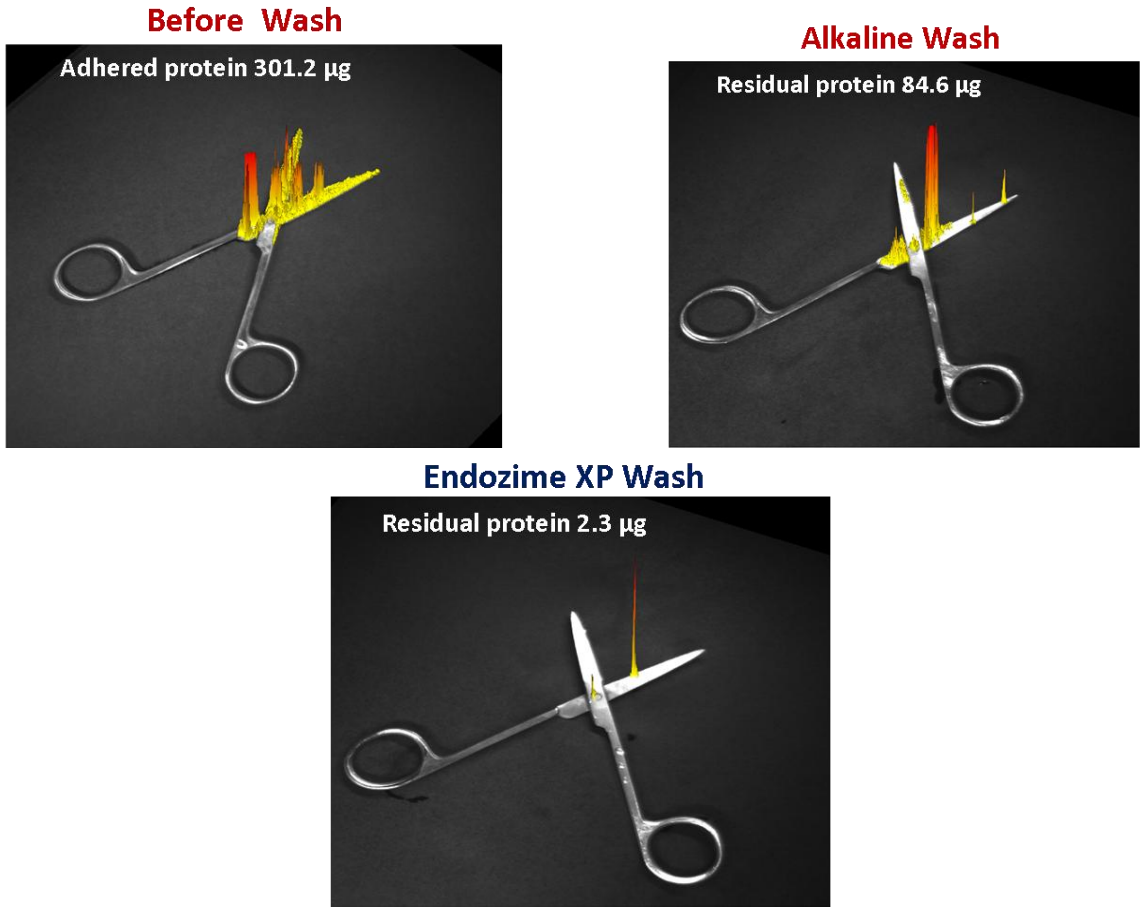


***Figure 3:** Typical fluorescent images of contaminated scissors washed in an SSD using Prolystica Alkaline (Bottom left) and Endozime XP (Bottom right). The pseudo colour from yellow to red depicts more protein in the red region.*

Figure 4: Typical fluorescent images of contaminated Forceps washed in an SSD using the Alkaline (Bottom left) and Endozime XP (Bottom right). The pseudo colour from yellow to red depicts more protein in the red region.

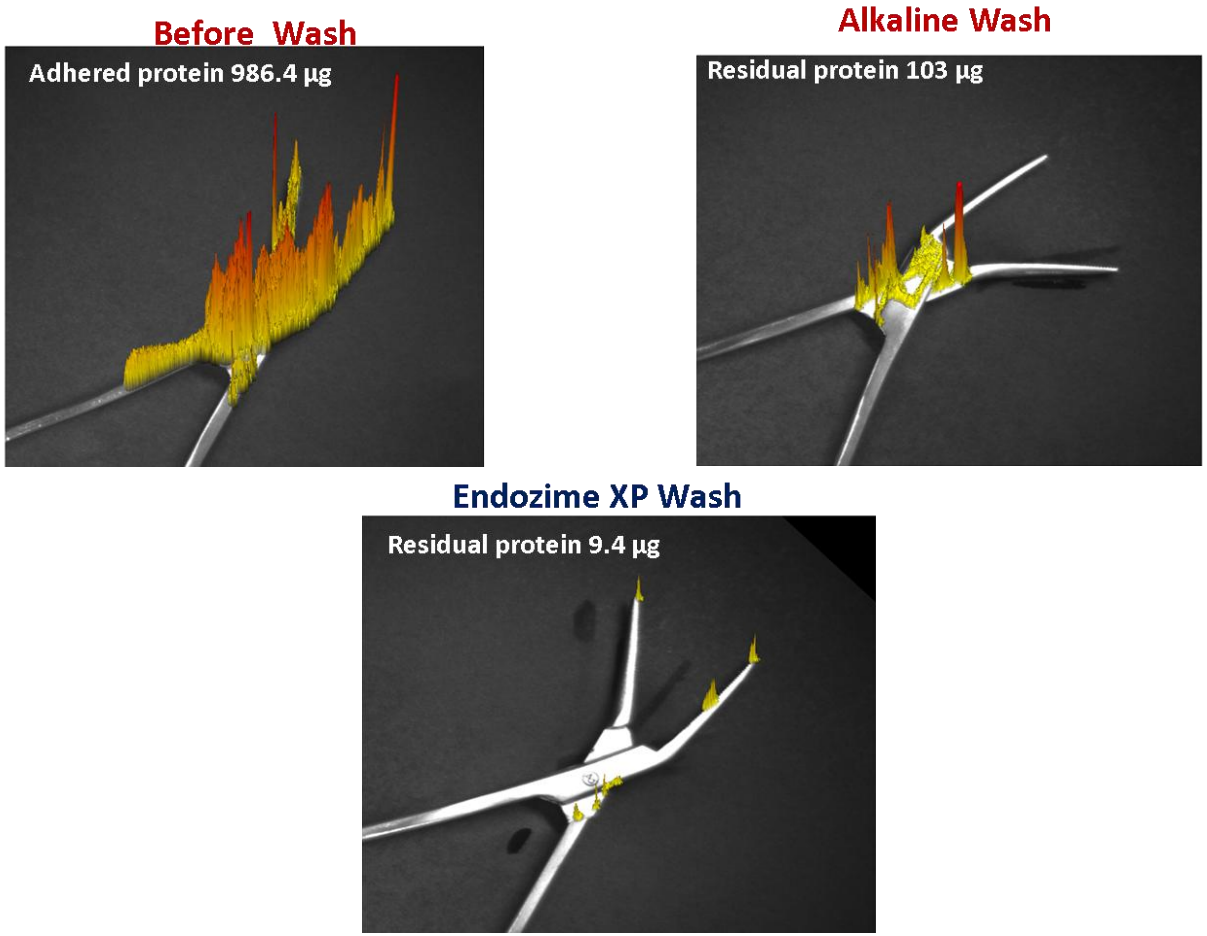


Figure 5: Typical fluorescent images of contaminated Spencer wells washed in an SSD using the Alkaline (Bottom left) and Endozime XP (Bottom right).The pseudo colour from yellow to red depicts more protein in the red region.

Conclusions:

The study revealed that a simple manipulation of brain tissue with surgical instruments results in about 33± 10 mg of tissue adhered to the working end. On average 369 ± 48 µg of total protein was present in the adhered tissue on each side of a forceps. When these instruments were washed with alkaline wash detergent in an SSD, 7.7 ± 1.6 % protein remained on the instrument, whereas instruments washed with Endozime XP had only 0.3 ± 0.2 % residual protein. This makes it 25 fold better in cleaning comparision. Scissors had 336 ± 28 µg total proteins in the adhered tissue and of this 13.9 ± 0.9 % protein remained after a the Alkaline wash. An EndoZime XP wash of these instruments had 2.3 ± 0.9 % protein remaining bound to the instrument surface with 6 fold better protein removal.

Hydrophobic proteins bind strongly to stainless steel and are hard to remove with just water. Protein when dried for more than 24 h further makes it difficult to remove protein firmly adhered to the surfaces. The use of proteolytic enzymes in detergents as in the Endozime XP wash detergent provides a better protein removal compared to alkaline wash detergents.

Although Endozime XP is better in removing protein, there are instruments with µg quantities of proteins still bound to the working end of the instruments (Figures 3,4 and 5). The use of sprays that keep the contaminated instruments moist (preventing the protein drying) would further improve the cleaning efficiency of Endozime XP.

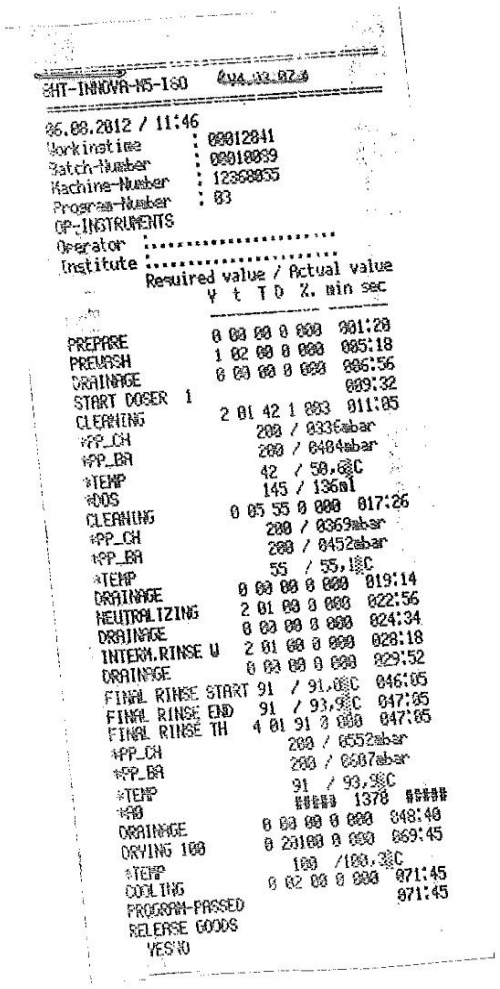
Recommendations:

1) Further testing of the cleaning efficiency of Endozime XP under moist conditions.

Appendix A:

Washer 1 (Used for Alkaline wash)

Washer 1



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06.08.2012 / 11:46
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Program-Number   : 03
OP-INSTRUMENTS
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Institute        : .....
                 Required value / Actual value
                    W  t  T D  X.  min sec
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PREWASH          1 02 00 0 000  005:18
DRAINAGE         0 00 00 0 000  006:56
START DOSER  1                  009:32
CLEANING         2 01 42 1 003  011:05
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*PP_BA             200 / 0404mbar
*TEMP               42  / 50,6°C
*DOS               145 / 136ml
CLEANING         0 05 55 0 000  017:26
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*PP_BA             200 / 0452mbar
*TEMP               55  / 55,1°C
DRAINAGE         0 00 00 0 000  019:14
NEUTRALIZING     2 01 00 0 000  022:56
DRAINAGE         0 00 00 0 000  024:34
INTERM.RINSE W   2 01 00 0 000  028:18
DRAINAGE         0 00 00 0 000  029:52
FINAL RINSE START 91  / 91,0°C  046:05
FINAL RINSE END   91  / 93,9°C  047:05
FINAL RINSE TH   4 01 91 0 000  047:05
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*TEMP               91  / 93,9°C
*H0                ##### 1378 #####
DRAINAGE         0 00 00 0 000  048:40
DRYING 100       0 20100 0 000  069:45
*TEMP              100  /100,3°C
COOLING          0 02 00 0 000  071:45
PROGRAM-PASSED                  071:45
RELEASE GOODS
  YES'0
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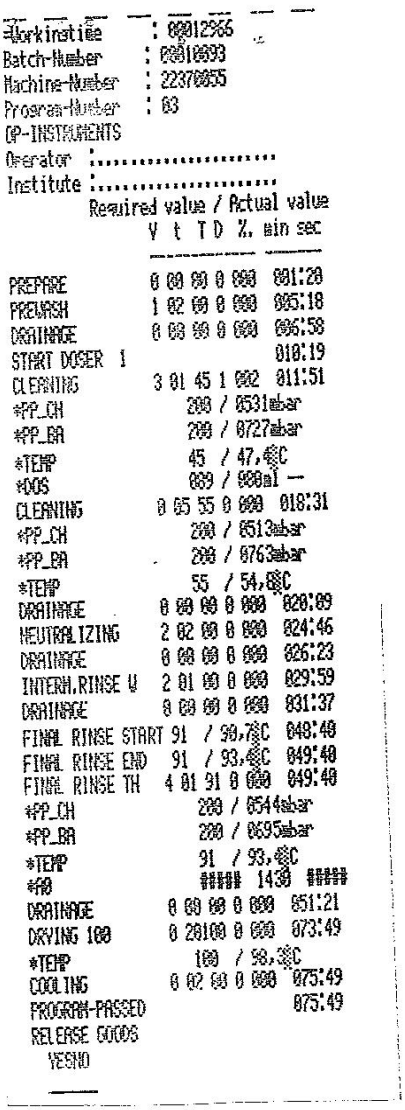
Washer 2 (Used for Endozime XP)

Washer 2



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Machine-Number   : 22370055
Program-Number   : 03
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Operator :.....
Institute :.....
          Required value / Actual value
                V  t  T D  %, min sec
                ---------- -------
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DRAINAGE        0 00 00 0 000  006:58
START DOSER  1                 010:19
CLEANING        3 01 45 1 002  011:51
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DRAINAGE        0 00 00 0 000  026:23
INTERM.RINSE U  2 01 00 0 000  029:59
DRAINAGE        0 00 00 0 000  031:37
FINAL RINSE START 91  / 90,7°C 048:40
FINAL RINSE END   91  / 93,4°C 049:40
FINAL RINSE TH  4 01 91 0 000  049:40
*PP_CH             200 / 0544mbar
*PP_BA             200 / 0695mbar
*TEMP               91  / 93,4°C
*A0                ##### 1439 #####
DRAINAGE        0 00 00 0 000  051:21
DRYING 100      0 20100 0 000  073:49
*TEMP              100  / 98,3°C
COOLING         0 02 00 0 000  075:49
PROGRAM-PASSED                 075:49
RELEASE GOODS
  YESNO
  ____
```

Appendix B:

STANDARD OPERATING PROCEDURE FOR "IN SITU PROTEIN DETECTION ON SURGICAL AND DENTAL INSTRUMENTS USING OPA-NAC SPRAY REAGENT AND ProReveal SYSTEM".

1. Make sure the USB cable and the 9 pin Serial port from the ProReveal are connected to the computer.
2. Turn on the computer and await the desktop to appear.
3. Turn on the ProReveal at the switch at the back of the box.
4. Open the Surgical Scanner software by double clicking on the software icon.
5. Click on the live button on the screen and make sure that the camera is working and you can visualise the interior of the dark room.
6. Open the door of the dark room and place a fresh Black sheet of paper provided.
7. At the beginning of each session place a new calibrant strip on the black paper in the centre of the ROI (a blue box on the screen)
8. Take the OPA-NAC spray bottle and make sure that there is enough spray reagent in the transparent bottle provided for a days work.
9. Lightly spray the reagent on to the Calibrant strip.

Note: Face mask could be worn if necessary while spraying the reagent

10. Close the door to the dark room
11. Press the scan button and wait for the white and UV light images to be captured.
12. Once the images are captured and the analysis is done. Ignore the traffic light signals.
13. Now repeat the procedure with each instrument to be accessed.

Note: Hold the non-working end and avoid contaminating the instrument with used or dirty gloves. Change gloves between instruments if necessary)

14. A traffic light signal will appear with a green, red or an amber light depending on the threshold settings set by the supervisor.
15. If red consult the supervisor.
16. All processed instruments must be rewashed.
17. Dispose of contaminated black paper.
18. The volume of the protein fluorophores is shown in the results section of screen.

Statement:

This study was conducted using the agreed protocol V020712 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 the 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 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

15/08/2012.