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Research Article

Protein Residue Removal Research Project University Hospital of Wales HSDU

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ABSTRACT

Over the last two decades, the Department of Health commissioned three National Research Teams (Southampton, London and Edinburgh) with a brief to investigate if protein residuals are a concern on reprocessed re-usable surgical instruments through a washer disinfector. The removal of biofilms or protein films from biomaterials is still a challenging task. In particular, for research investigations on real (applied) surfaces the reuse of samples is of high importance, because reuse allows the comparison of the same sample in different experiments. Burnt-on protein residues on the surfaces of the utensils used in dairy and meat processing industries possess a major challenge, where routine cleaning operations involve the use of hot alkali and/or acid, detergent washes followed by heat or chemical sanitization.

Keywords: Dangerous pathogens; Proteins; Surgical instruments

INTRODUCTION

Over the last two decades, the Department of Health commissioned three National Research Teams (Southampton, London and Edinburgh) with a brief to investigate if protein residuals is a concern on reprocessed re-usable surgical instruments through a washer disinfector. All three research teams developed a system to measure residual proteins on surgical instruments and all came to the same conclusion, that protein residuals were indeed present on processed instruments, in particular prion protein which has been proven to be extremely difficult to remove with current wash processes and chemistries [1]. On completion of the research, the Advisory Committee on Dangerous Pathogens (ACDP) Guidance was updated, which in turn pre-empted the update to HTM/WHTM 01-01. The updates recommended additional measures to combat residual proteins on surgical instruments. These were alternative detection systems for monitoring protein residuals on instruments (*in-situ* protein detection), reducing the time from patient to washer disinfector for high risk instruments, if there is a delay in reprocessing (preferably with the 6 hour time frame) systems must be place to keep instruments moist and finally protein based fully quantifiable process challenge devices should be considered as they come to market. Prions are easier to remove if they have not dried on the surface of the instrument, To enable efficient prion removal, theatre and SSD staff should ensure that medical devices are transported to the SSD for cleaning and reprocessing as soon as practically possible.

All of the recommendations of the revised HTM/WHTM 01/01 guidance have been implemented within the HSDU UHW following the release of the guidance including:

- Installation of *in-situ* monitoring protein detection system
- High risk sets being collected as soon as possible post-operatively and processed as priority on arrival in HSDU
- Implementation of an automated spray system to keep instruments moist for high risk, heavily soiled and time delayed sets.
- Implemented a trial of the fully quantifiable protein based PCD from Aseptium.

It was the trial of the protein based PCD, that instigated the research project which will be explained in this article [2].

BACKGROUND

Decontamination Manager Mark Campbell was approached by Aseptium to review the new protein based PCD following presenting at the CSC in Cardiff in 2018. The newly developed PCD fully meets the recommendations identified in the updated HTM 01-01 and a trial was agreed to monitor washer disinfector

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processes when challenged with a protein based material. It should be noted that at the time of this initial research, no standard or guidance was available for the manufacture of such PCD's. The PCD's were in development stage; therefore the results are for research purposes only. The PCD is a stainless steel token (SAE 316), impregnated with 1000 mcg of brain homogenate assay (ovine). The token replicates surgical instruments with residual protein residue present. In addition to the tokens, the system comes with a holder, which takes four tokens, with each position on the holder presenting the washer with different challenges [3]. These are: face up, face down, box joint and cannulated instruments (Figures 1 and 2).

Figure 1: Veritest.

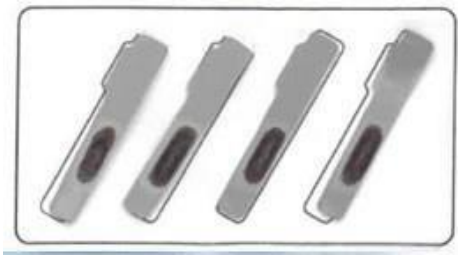

Figure 2: A representation of tumour components transported from a glioma into CSF and peripheral blood.

On arrival of the samples from Aseptium, a testing day was arranged to evaluate the PCD's, washer disinfector performance and review *in-situ* monitoring effectiveness on measuring the residuals on the processed tokens.

A washer disinfector was selected and the programmed wash cycle was agreed for use as this cycle is predominately used on all of the 9 washer disinfectors in HSDU, which includes:

- Pre-wash 5 min below 30°C
- Wash 10 min 60°C
- Rinse 2 min 60°C
- Disinfection 90-93°C 2-3 mins
- Drying 15 min

The chemistry on the washer disinfector, was an alkaline detergent pH 10 in the wash cycle. As this was a cleaning efficacy test, the cycle was aborted following the rinse in line with the cleaning efficacy test guidance in WHTM 01-01. On completion of the test cylescycles, the PCD's were removed from the washer disinfector, and on visual; inspection all four tokens had visual protein residues present. The chemistry and wash cycle had no effect on the removal or break down of the protein challenge.

The washer disinfector was fully validated in line with the recommendations in WHTM 01-01, with weekly, quarterly and annual test performed with no issues identified. These results were alarming and highlighted that the washer disinfector

Chemistry can achieve excellent results when faced with soil based challenges (Browne Soil or Soil Based PCD's), however we are seeing ineffectiveness at removing residual proteins. This initial test instigated the research project, with the Sterile Service Manager asking the question, "Can we optimise the decontamination process /chemistry, to effectively pass the protein based challenge", not "this PCD is too difficult, we won't use it", which may have been the response of other users.

RESEARCH PROJECT

Mark Campbell initiated a research team, to evaluate the use of alternative chemistry types, washer disinfector cycles and decontamination processes to determine the efficacy of brain homogenate removal from a PCD. The research team included the decontamination service manager, decontamination scientist, decontamination specialised engineer and representation from the chemical manufacturer. The initial tests were to monitor the performance of hybrid chemistries (mild alkaline/enzymatic) on the removal of residual proteins. Previous research (Perrott/Keevil) identified that enzymatic' s are more effective on the removal of residual proteins, than neutral/alkaline detergents [4]. The thought process was with the use of alkaline detergents excellent results werebeing received on soil tests, so with the combination of alkaline andenzymes would we see improved results on the protein challenge.

Test 1

A testing day was arranged and the washer disinfector was primed with a mild alkaline enzymatic and set at dosing 4 ml/litre. The wash cycle parameters were not changed from the initial test, to confirm if alternative chemistry types would make a difference. Again a soil test cycle was deployed with the cycle being aborted after the rinse. The first test, again showed failures on all four PCD's with the alternative chemistry having no effect on breaking down the residual soil (Figures 3 and 4).

Figure 3: A representation of tumour components transported from a glioma into CSF and peripheral blood.

Figure 4: A representation of tumour components transported from a glioma into CSF and peripheral blood.

The chemistry manufacturer requested increasing the dose for the second test, which was implemented at 5 ml/litre. Again failures on all four tokens were received. A final test was agreed, dosing at 6 ml/litre and reducing the wash temperature to 45° C, in an attempt to optimise the performance of the enzymes. The final test did show a slight breakdown of protein on token three, however all four tokens presented a failed result.

Test 2

The second test was arranged to evaluate if a hybrid chemistry from an alternative manufacturer would have an impact on the results. A second manufacturer was invited to take part in the research, with the same wash cycle and dosing deployed. Again poor results were received on the 5 cycles performed, regularly all four tokens failing the test (Figure 5).

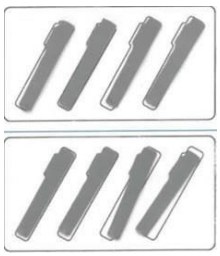

Figure 5: A representation of tumour components transported from a glioma into CSF and peripheral blood.

At this stage of the research, the Sterile Service Manager identified that the test being performed did not replicate the actually decontamination process deployed in the HSDU. Due to the time from patient to HSDU at the University Hospital of Wales, all visually contaminated instruments are manually pre-cleaned pre-washer disinfector. To replicate this process, it was agreed to soak the four tokens in a measured enzymatic solution for 15 mins,then put them through the washer disinfector to see if this had an impact on the results. On examination of the tokens following the soil test, all four tokens were visually clear and on measurement using the *in situ*-monitoring system, the average residue was 1.7 mcg (Figure 6).

Figure 6: A representation of tumour components transported from a glioma into CSF and peripheral blood.

These results pre-empted a discussion with the specialist engineer, asking the question "How can we replicate the pre-soak as part of the washer disinfector process?"

It was agreed to extend the pre-rinse to 15 mins below 30°C and dose this part of the cycle at 4 ml/litre with the enzymatic, this would replicate a manual soak/pre-clean through the sinks.

Test 3

The cycle was optimised with the additional phase and a soil test cycle commenced. On completion, the tokens were analysed, with significant progress being achieved (Figure 7). Repeated cycles with the pre-rinse deployed were performed with improved results (over 15 cycles), however there was still inconsistency, in particular on token two. The aim of the testing was to come to a process where we achieved consistent passes on all four test tokens.

Figure 7: A representation of tumour components transported from a glioma into CSF and peripheral blood.

RESULTS AND DISCUSSION

Previous research has indicated that ultra-sonic activity is very effective at breaking down residual proteins. On the bases of this, it was agreed to try a 20 min ultra-sonic cycle (dosed with mild alkaline enzymatic) pre washer disinfector. Following the sonic cycle, the tokens were visually inspected, with significant removal of the residual protein observed. The test device was then put through the optimised washer disinfector, and on completion all four tokens were visually clear [5]. *In-situ* monitoring testing confirmed 0 ng residual on all four tokens. This was the first time that a consistent pass had been achieved, with no residual protein present. With the implementation of the ultra-sonic stage continual testing has been performed and to date over 30 cycles have been completed (120 tokens), with all tokens consistently achieving 0 ng. It has proven that with optimisation of process, chemistry and parameters we can effectively remove residual proteins from surgical instruments. It should be noted, this initial research project has been limited to the volume of PCD's used [6]. Many more PCD's will be required to establish consistent and reliable results as the project develops, to include the placement of the PCD blocks in various positions within the WD chamber and alternative decontamination equipment. Conclusions returned can only be verified with chemical solutions used under this test, other products may produce differing results for the single or two stage process when tested using identical methodologies (Figure 8).

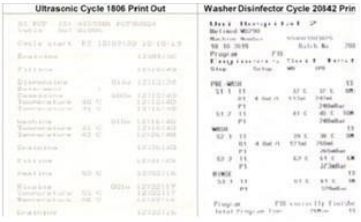

Figure 8: A representation of tumour components transported from a glioma into CSF and peripheral blood.

CONCLUSION

The next question for the research group is to investigate if human brain protein behaves the same way as the ovine protein being used for the testing to date. A research group has been agreed with representation from Cardiff and Vale Health Board, Aseptium, Glasgow University and Medical Device Manufacturers to asses further the process in use, to gauge a greater understanding of what is actually happening to the protein during reprocessing. The second half of the study will be to produce similar tokens with human brain homogenate with elevated level of amyloid protein (cause of Alzheimer's disease) and asses the effectiveness of the process on human proteins. This will be a 3 year project, with a PhD student deployed to publish the findings. It should be noted that previous research has only proven de-activation of prion, potentially with this project we will be able to prove we can effectively remove prion with effective optimisation of decontamination processes. If proven successful, the aim of the wider research group will be to present the findings to the DoH/NICE for a potential revision of guidance, to include optimisation of WD cycles/ decontamination processes etc.

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