Improved surveillance of surgical instruments reprocessing following the variant Creutzfeldt-Jakob disease crisis in England: findings from a 3-year survey.

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Running title: Improved surveillance in SSDs

**Summary**:

***Background:*** Sensitive, direct protein detection methods are now recommended for the inspection of reprocessed reusable surgical instruments in England to reduce the risk of prion transmission.

***Aim:*** To implement an established, highly sensitive methods to quantify proteinaceous residues on reprocessed instruments in a Sterile Services Department and evaluate its potential impact on service provision.

***Methods:*** We introduced highly sensitive epifluorescence (EDIC/EF) microscopy in a large SSD. Over three years, we periodically tested two models of washer disinfector using stainless steel tokens spiked with mouse brain homogenate or Browne test soil for comparison. We also obtained data and feedback from staff who have been using EDIC/EF to examine nearly 3,000 reprocessed instruments.

***Findings:*** All reprocessed test surfaces harboured residual contamination (up to 258.4 ng from 1 microgram spikes). Proximity between surfaces affected decontamination efficacy and allowed cross contamination. Up to 50 ng *de novo* proteinaceous contamination was deposited on control surfaces after a single AWD cycle. The test soil behaved differently than real tissue contamination. SSD staff observed proteinaceous residues on most reprocessed instruments using EDIC/EF, which can detect far smaller amounts than the currently accepted national threshold of five micrograms per side.

***Conclusion:*** Implementing recent national guidelines to address the prions concern proved an eye-opener. Microscopic levels of proteins remain on many reprocessed instruments. The impact most of these residues, potentially including prions, may have on subsequent patients after sterilization remains debatable. Improving surveillance capability in SSDs can support decision making and raise the standards of surgical instruments reprocessing.

**Keywords:** Surgical instruments, sterile service departments, surveillance, proteins, prions

**Introduction**

Millions of surgical instruments are reprocessed daily in Sterile Services Departments (SSDs) worldwide following widely accepted decontamination protocols which have not significantly evolved in the past decades. After manual pre-wash, complete instrument sets employed in various surgical procedures are placed together in automated washer disinfectors (AWDs) and reprocessed following a validated cycle (Fig. 1A). SSD staff rely on the recommendations from chemistries and equipment manufacturers, and AWD self-generated reports after each cycle. Macroscopic residues are sometimes detected and instruments washed again, causing delays. Instruments deemed clean after inspection are repacked and subjected to heat sterilisation by autoclaving. Staff handle instruments with bare hands in the “clean area” of many SSDs, suggesting confidence in both the previous automated reprocessing and subsequent autoclaving, although evidence suggest this practise might have detrimental effects.1, 2

Following the variant Creutzfeldt-Jakob disease (vCJD) crisis in the UK at the turn of the century, emphasis was put on improved surveillance of reprocessed surgical instruments. While the fear of a new wave of vCJD did not materialize to date, assessing the efficacy of reprocessing remains a key objective of sterile service departments (SSDs).3-12 Over the last 20 years the decontamination industry has developed new chemistries claimed to reduce the specific risks posed by prions.8 There is little evidence of the impact, if any, these have had on patients’ safety. This is due to vCJD variable incubation period linked to patients’ genotype, the absence of reliable pre-clinical tests, the limited number of identified and reported cases of surgical transmission and the difficulty to quantify specifically any prion infectivity remaining on reprocessed instruments. Following a precautionary principle, instruments used on tissues deemed at risk in identified CJD or vCJD patients are often removed from common use and patients who have been in contact with those instruments become themselves “at increased risk” of developing and transmitting the disease.13

Current surveillance of instruments carried out between the AWD and autoclaving stage relies on macroscopic, indirect and often subjective assessments. The sensitivity of kits relying on swabbing or elution appears limited, 14, 15 although these are designed to achieve the currently accepted levels of residual soil which are themselves dictated by the capability of commercialised cleaning and detection technologies. Consequently, the presence of residual contamination is often tolerated, though the recommended limits vary between countries or even clinical facilities.

In 2013 the Department of Health and Social Care introduced the Health Technical Memorandum (HTM) 01-01, “Management and decontamination of surgical instruments (medical devices) used in acute care”,16 which included recommendations for the use of direct, *in situ* protein detection methods in England. We previously developed highly sensitive episcopic differential interference contrast microscopy combined with epifluorescence (EDIC/EF) to quantify various surface contaminants.8, 17, 18

The aim of this study was to evaluate the implementation of EDIC/EF for the daily inspection of reusable surgical instruments and periodically testing AWDs, comparing the use of a test soil with real tissues.

**Methods**

*Preparation of test tokens and selection of sample instruments*

Polished 316L stainless steel tokens (standard microscope slide size) perforated at both ends for fixation during testing were cleaned to pristine condition by wiping and immersion for 10 minutes with acetone (Sigma, UK), followed by enzymatic cleaning (Enzol, Johnson & Johnson, US) according to the manufacturer’s instructions, a final rinse with deionized water and drying. Prepared tokens were subsequently spiked with the equivalent of 1 µg of total protein from a healthy (“normal”) mouse brain homogenate (NBH, Pirbright Institute, UK; 1 µl of a prepared solution at 1 mg/ml in PBS; protein content determined using the Bradford method) or 1 µl of Browne soil (BWS; Medisafe, UK), both pre-warmed at 37ºC. The contamination spikes were left to dry and were transported to the SSD in a sealed box. Tokens were handled with nitrile gloves throughout experiments to prevent further contamination.

Surveillance of reprocessed instruments was carried out daily by SSD staff trained in the use of EDIC/EF. The study was approved by an independent local committee.

*Testing of AWDs*

Five tests were performed on two different AWDs (AWD 1 was a Steris Vision and AWD 2 a Steris Synergy), with approximately six months interval between tests to mimic the current testing frequency and exclude the potential impact of various environmental factors for a given short period of time. Tokens were attached horizontally at various positions and orientations to the four shelves of each AWD loader using plastic cable ties prior to loading instrument trays, on the same days for comparable cycles (Fig. 1B and 1C). For each shelf, the spiked surface of tokens was facing either upward or downward. Some spiked tokens were attached together with a pristine token so that the spiked surface was partly masked (“shadowing”) to assess the impact of masking on cleaning efficacy and potential cross-contamination of adjacent surfaces. The attachment was loose enough to allow water circulating through a 1 to 2 mm gap, as observed in instrument trays. Single pristine tokens were also placed on each shelf to assess potential *de-novo* contamination of clean surfaces during the cycles. We used a number and colour coding system (Fig. 1D) throughout the study to identify tokens in all subsequent data output.

*Staining and microscopy*

Residual proteinaceous deposits were stained with SYPRO Ruby (SR; Molecular Probes, Eugene, OR, USA) applied for 15 minutes at room temperature prior to rinsing with deionised water and drying. Fluorescence was detected by episcopic differential interference contrast microscopy combined with epi-fluorescence (EDIC/EF; Best Scientific, UK).17 Positive signal was quantified using Image Pro software (MediaCybernetics, Silver Spring, MD, USA). Clinical staff also followed this protocol for the routine measurements of total residual proteins on reprocessed instruments. We occasionally used our dual staining procedure, where thioflavin T (ThT) is applied for 10 minutes (at 0.2% w/v in hydrochloric acid 0.01M; followed by PBS then deionised water rinsing) prior to SR staining to distinguish amyloid-rich proteins among other contaminants.18

*Statistical analysis*

Measurements of residual soil on test tokens following AWD standard cycle were compared between AWDs, cycles and positions of test tokens within each AWD using SPSS. One-way ANOVA and Tukey’s range test were used for multiple comparison of the effect of orientation and shadowing within each AWD. Unpaired two-tailed t-test was used for pairwise comparison for soil and positions between AWDs. The Spearman’s rank correlation coefficient was used to test apparent trends. A value of P≤0.05 was considered significant.

**Results**

*Periodic monitoring of AWDs*

Using EDIC/EF we detected proteinaceous residues on all spiked test tokens following standard AWD cycles. The residual contamination profiles from NBH directly exposed to the water flow remained in the nanogram range for all positions tested in both AWDs in five separate tests over a period of 30 months (Fig. 2). Multiple comparison analysis within each AWDs pointed at two incidents where cleaning efficacy was reduced throughout the whole chamber in the first test in AWD 1 (p=0.0005) and the second test in AWD 2 (p=0.005).

A more recent AWD model, AWD 1, consistently achieved a 2-Log reduction with test tokens spiked with 1µg NBH (Fig. 3A) and nearly 3-Log reduction on some tokens spiked with 1µg BWS directly exposed to the water flow (positions 1-8; Fig. 3B). One soil/position combination consistently showed a reduced cleaning efficacy in AWD 1, namely NBH in position 10 (NBH on top side, shadowed by another clean token and placed on the second shelf from the top; p=0.02; Fig. 3A). The other AWD we tested in parallel (AWD 2) was an older model and appeared slightly less efficient at cleaning NBH than AWD 1, particularly with test tokens placed on the lowest shelves (1 to 2-Log reduction; Fig. 3D, positions 1-4), though differences between comparable positions within each AWD failed to reach statistical significance after five tests.

*Comparative results between real tissues and test soil*

We regularly measured lower amounts of residual proteins from the test soil after reprocessing in either AWDs when compared with real tissue,with less than 10% removal in some cases. Occasionally we observed spreading of the test soil constituents between the spiked and masking surface, with most of the soil still present (positions 9-16; Fig. 3B and 3E).

Since both machines showed no significant differences in cleaning efficacy between shelves (with the exception of position 10 in AWD 1), we grouped the data for each AWD according to orientation and shadowing (or not) only. This revealed that the orientation had no significant impact, but shadowing had overall a negative impact on the removal of NBH “on top” in both AWDs (Fig. 3C and 3F; positions 9-12) and NBH “below” in AWD 2 (Fig. 3F, positions 13-16). Shadowing also significantly affected the removal of BWS “on top” in AWD 1 (Fig. 3C, positions 9-12), but not in AWD 2 despite sporadic cases of poor cleaning mentioned earlier. Shadowing also led to significant levels of soil transfer to all initially pristine adjacent surfaces in both AWDs (Fig. 3A-F; positions 17-24).

*De novo contamination on surfaces*

We detected contamination on initially pristine surfaces after exposure to a single AWD cycle. There were no significant differences between shelves or orientations within each AWDs, so the data are presented as combined in Figure 3C and 3F. After each cycle in AWD 1 we measured 1.54±0.49 ng and 1.13±0.56 ng of protein deposited above (positions 1-4) or below (positions 5-8) the surface of test tokens (Fig. 3C, hatched columns). In AWD 2 these levels of *de novo* contamination were significantly higher than in AWD 1, amounting to 46.03±6.14 ng and 25.89±2.77 ng, respectively (Fig. 3F, hatched columns). The area examined was the same size as that examined on spiked tokens to facilitate comparison.

*Findings from routine surveillance of reprocessed instruments by SSD staff*

Staff at UHS Sterile Services Department examine nearly 3,000 instruments yearly using EDIC/EF. This technology easily allows the observation of 1 µg spikes we applied on tokens to test AWDs (Fig. 4A) and residual contamination can be detected down to the sub-nanogram range (Fig. 4B). Using bright light showed aggregates at interfaces between articulated parts (hinges) and between instrument’s teeth when present (Fig. 4C-N). On some occasions we performed our ThT/SR dual staining and observed amyloid and other proteins on the surface of some older instruments with microscopically damaged surfaces (Fig. 4k).

From several month surveillance by different observers, an order of “difficult” instruments could be established. This did not seem to be linked to any known bias towards particular instruments as suggested by the number of instruments examined for each type (Fig. 5). Examined surgical instruments harboured residual proteins in the range of hundreds of nanograms, with occasional peaks exceeding 1 microgram per instrument side (Fig. 6).

**Discussion**

Periodic tests on two AWDs and daily monitoring by clinical staff using EDIC/EF showed that AWDs’ overall efficacy varies from day to day and most reprocessed instruments retain microscopic contaminants on their surface. These residues remain undetected in SSDs using quality control tools with limited sensitivity.8 A test soil commonly used to monitor AWDs appeared easier to remove when directly exposed to washing but presented a greater challenge when shadowed by another surface, a situation frequently encountered in instrument trays.

The relatively high levels of residual soil we measured sporadically on some shadowed surfaces were reminiscent of occasional macroscopic residues reported by SSD staff. Besides the ongoing debate about the relevance of artificial test soils, our findings suggest that such tests may be more informative if they replicate the density of packaging of instrument trays, rather than using directly exposed flat surfaces.

Our findings raise questions regarding the risks associated with “historical” protein deposits on ageing reusable instruments. Hydrophobic molecules such as prions bind strongly to stainless steel surfaces, a characteristic which has been used in various prion infectivity studies.19-21 A study published in 2013 suggested an overall prevalence of 493 per million across the British population at the time.22 The implementation of drastic control measures in the food chain to prevent another “mad cow” crisis combined with the partial turnover of the UK population will have likely contributed to decrease that figure 20 years after the first vCJD peak. However, this prevalence figure theoretically remains correct for the part of the population old enough to have been potentially contaminated from the food chain, now ageing and increasingly requiring various medical treatments. Therefore despite limited evidence inherent to the difficulties to detect prions in asymptomatic individuals, the potential risk of surgical transmission of prions may remain greater in the UK due to the lack of relevant progress in decontamination methods and the long life of many reusable surgical instruments. A recent study reported prion infectivity in skin cells of patients affected with sporadic CJD, possibly from peripheral nerve tissues,23 yet transmission of CJD via peripheral tissues has never been reported. Fernie *et al.* have observed that autoclaving at 134ºC may not suffice to completely eliminate prion infectivity.24 Proteinaceous aggregates may facilitate the retention of embedded microorganisms. Other biologically active molecules such as bacterial endotoxins may also resist standard sterilisation.7, 25, 26 The impact of potentially immunogenic residues may be difficult to distinguish from the systemic response to surgical intervention, and as such may be under-reported.

The occasionally reduced overall cleaning efficacy we measured in two different AWDS was unlikely artefactual since these occurred at different dates while the AWDs were being tested in parallel (i.e. the test soils and preparation were the same). More tests might have improved the statistical significance of our findings, though our observations clearly showed that the AWDs tested rarely achieved the complete removal of tissue or test soil from new polished test surfaces. The quality of instruments surfaces is also likely to impact on cleanability. Findings reported here are from a single SSD that implemented EDIC/EF technology. While the surveillance of reprocessed instruments is ongoing and similar tests will be carried out on newer machines, other groups have tested different technologies based on direct epifluorescence detection.27, 28

The English Department of Health and Social Care now recommends the use of such direct *in situ* detection methods for the detection of protein residues on reusable instruments, with an acceptable level of total residual proteinaceous contamination initially set at 5 micrograms per instrument side.16 Considering an optimistic 20% recovery rate with swabbing, this is equivalent to the 1 microgram sensitivity claimed by the Ninhydrin test. In other words, this is a gentle transition towards more stringent controls. The reason for sporadic, poorer results remains difficult to ascertain. We previously reported how some cleaning chemistries may actually purify the amyloid-rich residues on contaminated surfaces down to levels undetectable using indirect methods based on elution or swabbing. Total protein measurements should be as sensitive and/or specific as possible to limit the risk of missing the most relevant residues, such as strongly bound hydrophobic prions.

The technology introduced here allows to re-examine the observations (and potentially verify measurements) thanks to the database of microphotographs, which may be used to refine reprocessing and instrument handling protocols. This should allow SSD staff to monitor more critically their processes and potentially stimulate the decontamination market to produce more performant solutions to the problem of residual microscopic soil on all reusable instruments. Despite its relative complexity, the system used for this study was easily adopted by SSD staff who had no handling issues. Such technologies can be tailored to the end user's needs and simplified to offer integrated "fool proof" tools. The manufacturer of EDIC/EF has now developed a simplified version with adaptations specifically designed for examining instruments in SSDs. The development of non-toxic and more specific markers for potentially infectious contamination such as protease-resistant prions may also contribute to establishing safer benchmarks for reprocessing protocols.

**Conclusions**

While possibly daunting at first, new technologies far more sensitive than colorimetric swab tests can be easily adopted in the routine function of SSDs. Readily implementable, better quality control tools made available to end users can ultimately allow better informed decision making at local and national level and improve patient safety. The findings reported here warrant maintaining precautionary measures whenever prion contamination is a known risk.

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**Declaration of interest**: None.

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**Author contributions**: RCH designed the study, performed AWD tests and analyzed results, trained SSD staff in the use of EDIC/EF microscopy, cured the data and drafted the manuscript; JH overview the use of EDIC/EF in SSD, helped with data curing and reviewed the manuscript; CWK helped with the design of the study and reviewed the manuscript.

**Data and materials availability**: Several thousand high definition micrographs of various instrument surfaces with proteinaceous residues available on demand.

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**Figure legends:**

Figure 1. A) Typical load showing different trays, each containing dozens of instruments tightly packed; B and C) Tokens held in position (positions 1 and 9/17 are shown) on an AWD cart before the loading of instrument trays; D) Colour and number identification of areas examined on test tokens placed on the four shelves of the AWD carts. Green (positions 1-4) or red (positions 5-8) denote soil spike, respectively, on top or below test tokens directly exposed to the washing chamber. Yellow (positions 9-12) and amber (positions 13-16) denote similar soil spike, respectively, on top or below the test token, shadowed by another token. Blue (positions 17-20) and purple (positions 20-24) denote cross-contamination on shadowing token above or below spiked surface, respectively.

**Figure 2**. Profiles from five cycles performed several months apart showing residual NBH contamination on test tokens directly exposed to the washing chamber in AWD 1 and AWD 2 (Positions 1-8). Green or red denote soil spike, respectively, on top or below test tokens attached to four shelves and directly exposed to the washing chamber. Tukey’s multiple comparison test showed an overall reduced efficacy in tested cycle 1 of AWD 1 (p=0.0005) and tested cycle 2 in AWD 2 (p=0.005).

**Figure 3**. Detailed efficacy tests of two AWDs. Profiles of residual normal brain homogenate (NBH) and Browne soil (BWS) contamination in AWD 1 (**A** and **B** respectively) and in AWD 2 (**D** and **E** respectively) from tokens set at various positions within each AWD. **C** and **F** show same profiles as well as *de novo* contamination in AWD 1 and AWD 2 respectively, after combining all shelves (i.e. considering only orientation and shadowing). Data show means ± SEM on a logarithmic scale, from five separate experiments. Colour code according to Figure 1D, hatched bars denoting *de novo* contamination on the superior (position 1-4) or inferior (position 5-8) side of exposed tokens. $: p≤0.05 from multiple comparison test between positions within each AWD. “+” and “++”: p≤0.05 and p≤0.01, respectively, when comparing shadowed and directly exposed surfaces for the same soil and AWD; “-” and “--”: p≤0.05 and p≤0.01, respectively, when comparing between NBH and BWS in otherwise same conditions within each AWD; “\*” and “\*\*”: p≤0.05 and p≤0.01, respectively, when comparing same soil and condition between AWD 1 and AWD 2 (symbols only shown on AWD 2 graph).

**Figure 4.** Residual soil on test and instrument surfaces seen under EDIC/EF. Example EDIC/EF scans (multiple pictures) of **A**) 1 µg NBH spike and **B**) 1.06 ng residual NBH on test tokens; **C**-**J**: individual microphotographs taken by SSD staff showing the amber SR-stained proteins and the same picture merged with the corresponding instrument stainless steel surface (**C** and **D**: osteotomy guide; **E** and **F**: skin hook; **G**-**J**: excavator); **K**-**N**: merged pictures showing proteinaceous residues (stained amber by SR) on the surface of various reprocessed stainless steel instruments (**K**: artery forceps; **L** and **M**: scissors; **N**: suction irrigator). Microphotograph **K** also shows ThT-stained amyloid proteins (blue) deposited near visible cracks on these ageing artery forceps (illumination was adjusted to facilitate visualisation of the blue on this merged picture). These instruments all passed the Ninhydrin test. Bars are 100 μm, except for scans as indicated.

**Figure 5.** Profile of residual contamination on some of the instrument types examined under EDIC/EF during this study. These instruments all passed the Ninhydrin test and were deemed ready for reuse (after sterilisation) according to current recommendations (5 µg residual proteins per instrument side). Data show means ± SEM, with the number of corresponding instruments observed in brackets.

**Figure 6.** Example of monthly profile of residual contamination on instrument routinely examined under EDIC/EF. Two scales are used for comparison. One instrument was found to have nearly 2 μg of residual proteins following reprocessing in AWD 5 on the 21st of that month (peak circled in red), during which six AWDs were in use. Some pictures from that instruments are presented, showing protein residues stained by SYPRO Ruby (bars are 100µm). All machines satisfied the current requirement of a maximum of 5 μg of residual proteins per instrument side.