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Technical note

The recovery of semen from bathwater using the Evidence Recovery System (ERS)

H. Page^{*}, A. Sarna¹, L. Watts², E. Ward, C. Hodgson, M. McKenzie³

Centre for Forensic Investigation, School of Science and Engineering, Teesside University, Borough Road, Middlesbrough TS1 3BA, UK

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ABSTRACT

Sexual offences are under-reported and ascertaining accurate offence numbers is difficult. Any methods which could increase the ability to obtain biological evidence or reduce the additional distress associated with reporting a sexual offence may result in an increase in reporting this crime type. The Evidence Recovery System (ERS) is designed to collect trace evidence, including hairs, fibres and biological evidence, from bath or shower water in a non-invasive manner. Initially, samples of semen were placed in baths filled with water, and washing was simulated using a range of body wash products. The water was then drained through the ERS before its filters were subjected to acid phosphatase testing and haematoxylin and eosin staining of spermatozoa. Recovered spermatozoa were then graded accordingly. Following this, the experiment was repeated with the addition of dirt/dust particulates during the washing stage, to simulate recovery of biological evidence in a more realistic environment. The results showed that spermatozoa considered 'easy to find' could regularly be obtained from bathwater using the ERS. It appeared that this recovery was not affected by the presence of different body wash products. When dust/dirt particles were added, the number of spermatozoa recovered increased at two of the evidence collection stages. The difference in recovery was considered to be statistically significant. This study provides evidence to suggest the feasibility of use of the ERS as a method to collect semen evidence from individuals subjected to sexual offences. The recovery of spermatozoa does not appear to be affected by the presence of a body wash, but does appear to be improved when skin cells, hair and other debris are transferred into the water, as would be likely during a bath/shower. Further to this, the possibility of obtaining spermatozoa from the home bath or shower of a victim following a post-offence bathing experience is implied.

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1. Introduction

In 2010/11 the total number of sexual offences recorded by the police in England and Wales was 54 982. This was a 1% increase on the previous year [1]. The nature of sexual assaults usually means that victims are, to varying extents, psychologically traumatised [2] resulting in a level of under-reporting for this crime type. It is difficult, therefore, to make accurate and reliable assessments of the available data due to the effect of under-reporting. Consequences of the psychological trauma for the victim, include the need to try and cleanse themselves by washing or douching [3] and/or not wishing to undergo a full medical

examination. This is due to the strong incident-related fear cues associated with the examination [4]. Both of these result in a vastly reduced chance of recovering forensic evidence.

The Evidence Recovery System (ERS), from Forensic Rescue Ltd., is a simple device which has been designed as a non-invasive method of collecting physical evidence from bath/shower water, including hairs, fibres and biological evidence. The ERS uses a 100 µm single-layered microfilter (the Forensic Rescue™ Plug) to collect a range of trace evidence types including fibres, debris and biological material such as semen. The ERS kit contains three plugs, each fitted with a microfilter. Each plug serves a function within the evidence recovery method to act as; (1) a control, (2) sieving the bath/shower water and (3) sieving the particulates which are rinsed from the side of the bath. The fourth stage is to wipe down the sides of the bath using the sterile wipes provided in order to capture any particulates which may remain on the side of the bath after the rinsing stage.

There is little published research available regarding the recovery of evidence from bathwater or a bath after drainage, possibly due to the belief that the evidence may have simply been washed down the drain. Initial research carried out at Anglia Ruskin University [5] showed clearly that natural and synthetic fibres, head hair, pubic hair, glitter and vegetation are all likely to remain on the surface of a bath after the water

Abbreviations: ERS, Evidence Recovery System.

* Corresponding author. Tel.: +44 1642 384464; fax: +44 1642 342401.

E-mail addresses: h.page@tees.ac.uk (H. Page), Anna.Sarna@keyforensic.co.uk (A. Sarna), lucy.corner@hotmail.co.uk (L. Watts), emjaneward@btinternet.com (E. Ward), christina.hodgson@hotmail.com (C. Hodgson), marta.mckenzie@hotmail.co.uk (M. McKenzie).

¹ Present address: Key Forensic Services, Norwich Research Park, Colney Lane, Norwich, Norfolk NR4 7UH, UK.

² Present address: Pathology, South Tees Hospitals NHS Trust, James Cook University Hospital, Marton Road, Middlesbrough TS4 3BW, UK.

³ Present address: FujiFilm Diosynth Biotechnologies, Belasis Avenue, Billingham TS23 1LH, UK.

has drained. It was also shown that utilising the ERS facilitated the recovery of greater quantities of these physical trace evidence types.

These preliminary results suggest that the ERS could, potentially, be utilised in two manners for the collection of biological evidence. The first application would be for use as an alternative means of evidence collection if a victim does not wish to consent to a full forensic medical examination post-sexual assault. Having an evidence collection method available which is non-invasive is likely to pose less of a psychological trauma to the victim than the traditional forensic medical examination. The second application would be for use by scene examiners on a victims' home bath or shower where there is a case of delayed reporting by the complainant. Analysis of police rape and sexual assault files by Jordan [6] showed that 22% of 164 files examined were reported in a delayed manner. The ERS could be utilised for the purpose of retrieving evidence which may have adhered to the surface of the bath/shower after a victim has washed. Based on the preliminary study, it is predicted that trace evidence may remain present on the surfaces of the bath/shower and is not all lost down the drain following a washing episode.

This study aimed to determine whether or not it was possible to recover semen from bathwater using the ERS as a collection device. Further to this, the study aimed to ascertain whether the presence of body wash products or dust/dirt particulates had any effect on the recovery of spermatozoa using the ERS. In both simulations a clean bath was employed to mimic one of the application scenarios expected for the ERS i.e. a disinfected/clean bath/shower at a Sexual Assault Referral Centre (SARC) where an individual doesn't consent to a full forensic medical examination. Body washes, to simulate a victim washing themselves during the bathing process, were used to ascertain whether or not there was a negative effect on the identification of semen or the recovery of spermatozoa due to their cleaning abilities. Dirt/dust particles were added, in separate experiments, to represent a real-life scenario where an individual would take a bath and transfer skin cells, hairs and other debris into the water.

The persistence of semen varies according to the location of its deposition and the 'surface' on which it is found [7,8]. On clothing, Farnen et al. [9] found that garments stained with semen and then washed at 40 °C returned a negative acid phosphatase (AP) test but returned positive results for spermatozoa in 66% of clothing items. This is consistent with findings from Kafarowski et al. [10] and Jobin and De Gouffe [11]. In both instances, semen-stained pants were washed and positive spermatozoa identification was still possible. In the work by Jobin and De Gouffe [11], AP tests on the washed pants were negative and the number of spermatozoa identified on cotton pants was higher in comparison to those on nylon pants, indicating differential retention of spermatozoa. The water-soluble nature of the enzyme AP means that presumptive location and detection of semen using the AP test after wetting or washing has occurred, both on fabrics and on individuals, are affected. The size of the detected area is altered due to diffusion [12,13] and the detection ability is reduced and likely to return negative results in such instances where washing has taken place. Confirmation of semen, using staining methods for the identification of spermatozoa, is needed, even for those items considered negative for AP within this study.

2. Method

2.1. The Evidence Recovery System (ERS)

The ERS, from Forensic Rescue Ltd., 96 Staverton, Trowbridge, BA14 6PE, is composed of three colour-coded lidded plugs (red, green and blue) and a single SteriClean® XL dry filter wipe. The structure of the plugs can be seen in Fig. 1. The plugs are constructed of plastic with a medical grade, woven polyester, single-layered filter creating pore sizes of 100 µm.

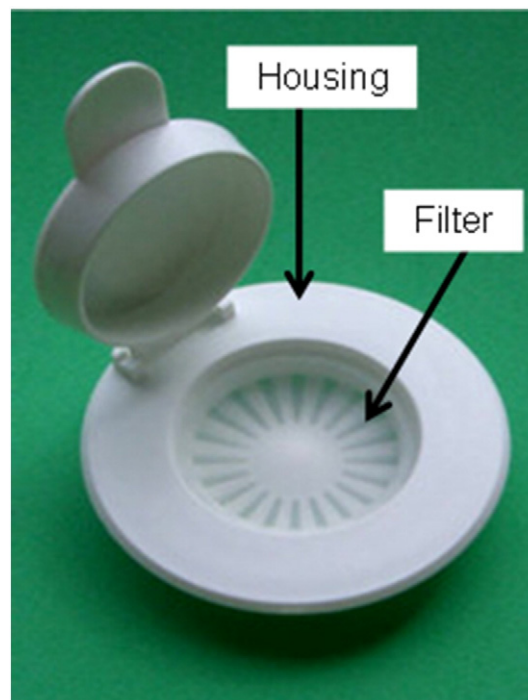


Fig. 1. An Evidence Recovery System (ERS) plug, showing the plastic housing, the lid and the medical grade, woven polyester, single-layered filter.

2.2. Recovery from clean baths – body washes

Before each use, the bath and area surrounding the bath were cleaned using a non-bleach-based domestic cleaner. The sides of the bath were rinsed with water using a hand shower. The inside of the bath was sprayed with Trigen™ solution disinfectant and left for 10 min.

Following the ERS utilisation guidelines [14], the first plug (red) was placed into the plug hole in an open position and the inside of the bath was rinsed for 3 min using a hand shower. Once the water had fully drained, the plug was removed and placed in its corresponding retaining tub. This plug is subsequently referred to as the "Control Plug".

Plug two (green) was placed, closed, within the plug hole. The bath was then filled with 120–130 l of water at a temperature ranging between 35 and 40 °C. Reference values for human semen characteristics [15] suggest that 90% of males produce between 1.5 ml and 6.8 ml of semen per ejaculate with a spermatozoa concentration ranging between 15×10^6 spermatozoa/ml and 213×10^6 spermatozoa/ml. Approximately 3–4 ml of spermic semen was added to each bath to represent this quantity. To simulate the victim washing themselves during this process, varying quantities of different body wash products were added to the bath water according to Table 1. Five bath runs were completed for each body wash product. The bath water was then thoroughly stirred before being left to settle for 5 min. A control bath, with no added body wash, was also completed for comparison purposes. A total of five repeats were completed for the control baths. The plug was opened, allowing the water to drain through the micro-filter. Once fully drained, the plug was carefully removed and placed

Table 1
Body washes and quantities.

Body wash type	Approximate quantity (ml)	AP testing method
Canesten® Care Feminine Wash	5	Filter swabbed
Simple™ Moisture Bath Cream	5	Filter swabbed
Dove® Beauty Care Body Wash	10	Direct test
Femfresh™ Wash	10	Direct test

into its corresponding tub. This plug is subsequently referred to as “Bath”.

The third plug (blue) was placed, open, into the plug hole. The inside of the bath was rinsed with water using a hand shower for approximately 2 min, as per the ERS method. The plug was then removed and placed into its corresponding tub. This plug is subsequently referred to as “Rinsing”. The sides and bottom of the bath were wiped using a single SteriClean® XL dry filter wipe, now referred to as “Wipe”.

For each bath run, the speed of drainage and any additional relevant observations were recorded.

2.3. Recovery from clean baths – adding dirt/dust particles

The experimental work was repeated as outlined in Method section 2.2, with a variation at the stage of plug two (green). In this work, body washes were not introduced into the bath water with the semen aliquot but instead approximately 1 g of dirt/dust particles was introduced. In order to represent the likely material transferred into bath water, from a person, during a bath, such as skin cells, hair and other debris, dirt/dust from a Hoover was utilised. AP testing was carried out using a direct test of the filter. Each bath run (clean water and dust/dirt particles) was repeated 20 times.

2.4. Presumptive acid phosphatase (AP) testing

Wipes were tested in a standard manner, by overlaying the wipes with dampened filter paper and with pressure being applied for 2–4 min. The filter paper was then sprayed with fast black AP reagent. Any colour change, and the time at which it occurred, was recorded.

The ERS filters were tested in two ways, as indicated in Table 1. One method was a direct test with moistened filter paper pressed on to the micro-filter. The second was by swabbing the micro-filter with a moistened swab tip. The tip of the cotton swab was cut off and placed in a spineroo with a few drops of water before the contents were mashed. The spineroo was centrifuged at 9 000 g for 3 min and the microtube containing the swab was removed. A small quantity of the eluate was pipetted on to a piece of filter paper before being tested with AP reagent. All ERS microfilters from the dust/dirt particles experimental runs were tested using the direct test.

2.5. Haematoxylin and eosin (H&E) staining for the detection of spermatozoa

A section of the ERS microfilter was cut into smaller squares. The tip of the cotton swab (from the filter swabbed AP samples) was cut off and the AP positive sections of the wipes were cut into smaller pieces.

For the ERS filters, swabs and wipes, the smaller cut segments were placed into individual spineroos. A small quantity (2–3 drops) of sterile distilled water was added to the microtube before the contents were mashed. The spineroo was centrifuged at 9 000 g for 1 min and the microtube containing the filter sections was removed. Using a pipette, the eluate was gently mixed to resuspend the contents and a small quantity was then placed on a glass slide. The stain was fixed by slow drying on a hotplate (set to approximately 50 °C). Additional drops of eluate were placed on the slide to build a film on the slide.

The slide was then stained using the H&E staining method. This involved flooding the stain with Formyl Alcohol before being left for 30 s. The slide was then briefly rinsed with gently running water, before Haematoxylin was added for 10 min. This was replaced with Acid Alcohol, and left for 30 s, before being replaced with Di-sodium hydrogen ortho-phosphate and being left for 1 min. This was then briefly rinsed with gently flowing water before Eosin was added for 30 s. This was then rinsed again with water. The slide was then dried before a cover slip was mounted using mounting media.

Table 2
Examination scale for spermatozoa counting. [16].

Scale	Description
Trace	Less than five spermatozoa
+	Hard to find
++	Some in some fields, easy to find
+++	Many or some in most fields
++++	Many in every field
T	Complete spermatozoa with tails present on some or all spermatozoa

Once dry, the slides were examined at 400× magnification. A standard scale (Table 2) was used to broadly grade the number of spermatozoa located on the microscope slides.

2.6. Statistical analysis of results

Statistical analysis of the results was performed using either the Kruskal–Wallis test or the Mann–Whitney–U test, as appropriate. *p* values are quoted.

3. Results

3.1. Recovery from clean baths – body washes

The results from the AP tests and subsequent H&E staining methods are summarised for each body wash type in Table 3.

One can see that the Control plug, when tested for AP, produced negative results as would be expected after the thorough cleansing of the bath. As these results were negative, H&E testing was not routinely completed on the micro-filters. However, as a checking mechanism some of the microfilters were processed and analysed for spermatozoa and all produced negative results.

Of all of the wipes taken from the sides of the bath, only three were found to be positive for AP, two when Simple™ Moisture Bath Cream was used within the bath and one when Dove® Beauty Care Body Wash was used. As with the Control Plugs, the remaining wipes were not routinely tested for spermatozoa. However just over half (15 in total) were tested further with H&E staining. Five of the wipes tested gave positive results for the identification of spermatozoa which were considered “hard to find”, and two were considered “some in some fields, easy to find”.

The AP results can be compared according to the method employed (Table 4). For Canesten® Care Feminine Wash and Simple™ Moisture Bath Cream the microfilter was swabbed and the swab subsequently tested for AP, whereas for the Dove® Beauty Care Body Wash and Femfresh™ Wash the microfilter was tested directly for AP. When the microfilter was swabbed, and the swab tested, only ten of the 20 tests returned a positive AP reaction. This is in comparison to 18 of 20 being AP positive when the microfilter was tested directly. The differences observed between these testing methods indicate that the direct test was the most successful at obtaining a positive AP result from the microfilter.

There was no significant difference ($p = 0.388$) between the AP results for the “Bath” plug. All body wash products and the control bath runs provided at least two positive AP reactions for the “Bath” plug but these were generally quite weak reactions. The H&E results produced from the “Bath” plugs showed that spermatozoa were captured on the microfilter. Median results for each body wash type were similar (++ and ++++) and when compared to the control. A range of results was obtained from these slides up to “++++, many in every field”. The results suggest that there was no significant difference in the ability to obtain spermatozoa when different body wash products were used, and at different quantities ($p = 0.916$).

The “Rinsing” plug results vary in a similar manner to the “Bath” plug in that positive AP results were found for all body wash products and the control runs. There was no significant difference ($p = 0.478$)

Table 3
Median spermatozoa counts, from five bath run repeats, utilising different body washes. For the AP reactions, –ve indicates a negative result, +ve indicates a positive result and (w) indicates a weak positive result. For the H&E results, the scale in Table 2 is employed.

ERS stage	Canesten® Care Feminine Wash		Simple™ Moisture Bath Cream		Dove® Beauty Care Body Wash		Femfresh™ Wash		Control	
	AP	H&E	AP	H&E	AP	H&E	AP	H&E	AP	H&E
Control Plug	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve
Bath	–ve	+++	+ve (w)	++	+ve (w)	++	+ve (w)	++	+ve (w)	++
Rinsing	–ve	++++	+ve (w)	+++	+ve (w)	++	+ve (w)	+	+ve (w)	++
Wipe	–ve	+	–ve	–ve	–ve	–ve	–ve	–ve	–ve	–ve

found. The H&E results show a slight decrease in the ability to obtain spermatozoa in comparison to the “Bath” plug for Femfresh™ Wash but an increase for Canesten® Care Feminine Wash and Simple™ Moisture Bath Cream. The maximum result observed for the “Rinsing” plug was “++++, many in every field”. There was no significant difference in the spermatozoa counts between body wash products ($p = 0.637$) for the “Rinsing” plug.

3.2. Recovery from clean baths – adding dirt/dust particles

The results for the AP tests and subsequent H&E staining are summarised in Table 5, for clean water and when dirt/dust particles were added.

All Control plugs were found to be negative for AP, which would be expected after the thorough cleansing of the bath. All Control plugs were tested using H&E staining, and no spermatozoa were found.

There was no significant difference ($p = 0.202$) between the AP results for the “Bath” plug when dust/dirt was added, in comparison to when the bath water was clean. When comparing the H&E staining results, the mode for both the clean water and the dirt/dust particles is the same at “++++, many in every field”. There was no significant difference in the spermatozoa counts ($p = 0.075$), although the p value lies at 7.5%, and a statistical difference may be observed if further repeats are completed.

The “Rinsing” results show that there was no significant difference ($p = 0.185$) in AP results when dirt/dust particles were added in comparison to when they were not present. When comparing the H&E staining results, the mode for the control bath was lower (++++) than when dirt/dust particles were added (++++). Statistical analysis shows that this is a significant difference ($p = 0.007$).

A total of eight of 40 wipes tested positive for AP. There was, however, no significant difference ($p = 0.107$) in the AP results for the “Wipe” when comparing clean water to added dust/dirt particles. All wipes that were tested for H&E were found to be positive for spermatozoa. There was a significant difference ($p = 0.001$) in the spermatozoa counts when comparing clean water (bi-modal +/++) and added dust/dirt particles (++++).

4. Discussion and conclusions

As one would expect, after thoroughly cleaning the bath with a non-bleach-based domestic cleaner, water and Trigene™ solution,

Table 4
Categorised acid phosphatase results, from five bath run repeats, utilising different body washes.

	Negative	Weak positive	Positive
Canesten® Care Feminine Wash	6	1	3
Simple™ Moisture Bath Cream	4	3	3
Dove® Beauty Care Body Wash	1	5	4
Femfresh™ Wash	1	5	4

no evidence of semen (via the AP test) or spermatozoa (via H&E slides) was found. This implies that the cleaning method used was sufficient to remove any previous traces of semen from the surfaces of the bath. These results would imply that the cleaning method utilised would be sufficient to use within a SARC shower/bath to remove traces of semen. However, based on the result of this research alone, one cannot be certain that all possible traces of DNA may be removed. Before utilisation within a SARC setting, the ability to obtain DNA profiles from external contamination after the “Control Plug” stage would require further examination.

Only 11 wipes tested positive for AP. This supports previous work by Farnen et al. [9], Kafarowki et al. [10] and Jobin and De Gouffe [11], who found that the water-soluble nature of the AP enzyme meant that negative results are likely in such instances where washing has taken place. In each of these studies, spermatozoa were still recoverable, despite negative AP reactions. The results obtained for the ERS also found this to be true. The levels of spermatozoa recorded varied considerably between trace levels and “++++, many in every field”. The quantity of spermatozoa recovered did appear generally to be lower when body washes were utilised but this difference was not significant. However, when dirt/dust particles were added the recovery of spermatozoa increased significantly. These results suggest that it would be feasible to consider attending the house of a victim who has recently showered there post-sexual assault, in order to wipe the sides of the bath to try and recover trace amounts of material. One would need to consider the reliability of this evidence collection method in conjunction with the likelihood of extraneous spermatozoa/DNA sources within the bath and the period of time between showing/bathing and collection. It is likely that quantities of spermatozoa and other trace evidence would be decreased further and external contamination increased if the shower/bath had been used in the interval. When assessing the wipes as a part of the ERS method collection, one needs to take into consideration the laboratory examination time in comparison to that of the microfilters. As it was not always possible to obtain a positive AP reaction, the selection of material to utilise for the subsequent H&E test was random. The authors believe that this contributed to the variability of H&E results obtained. Due to the success of spermatozoa recovery from the microfilter at the “Bath” stage and the “Rinsing” stage, it is possible that the “Wipe” stage of the ERS collection method is not required for optimum recovery of spermatozoa. This consideration should be viewed in conjunction with the ability of the “Wipe” stage to recover other trace particulates adhered to the bath

Table 5
Modal spermatozoa counts, from twenty bath run repeats, utilising clean water and adding dust/dirt particles. For the AP reactions, –ve indicates a negative result, +ve indicates a positive result and (w) indicates a weak positive result. For the H&E results, the scale in Table 2 is employed. Bi-modal categories are included where relevant.

ERS stage	Clean/control		Dirt/dust particles	
	AP	H&E	AP	H&E
Control plug	–ve	–ve	–ve	–ve
Bath	+ve	++++	+ve	++++
Rinsing	+ve (w)	+++	+ve	++++
Wipe	–ve	+ / ++	–ve	+++

sides and the reduction in cost to the price of the ERS with their exclusion.

The alternative methods used to test the microfilters for AP resulted in differences in the ability to obtain a positive result. The results show that the direct method (applying the filter paper directly to the microfilter) was more likely to obtain a positive AP result than the swabbing method. These findings concur with the work completed by Allard et al. [17] who determined that detecting AP on swabs seeded with various dilutions of semen was “best achieved” by a direct test. The swabbing method relies on additional water being added to the swab head and the suspension produced being tested, therefore resulting in an even greater dilution of any acid phosphatase present.

The dilution factor of the semen in the bath water should be taken into consideration when assessing the success or failure of the AP test. Two of the four bath products, some of the dirt/dust particle runs and the control baths, returned positive AP results. This is an encouraging result for assessing the sensitivity of the AP reagent used. As approximately 3 ml of semen was added to 120 l of water in this study, the dilution was 1:40000. The AP test has previously been reported to have a sensitivity of 1:40 [17]. Our own, in-house testing, has shown sensitivity levels reaching 1:20000 when the reagent is applied directly to the stain. The ability to obtain any positive AP results with the dilution factor used within this study suggests that the detection sensitivity of the AP test is increased due to all of the bath water and semen passing through the small surface area of the micro-filter (9 cm²), effectively acting as a concentration stage. Positive AP results were still obtained, albeit generally weaker, when additional water flowed through the microfilter as observed when examining the “Rinsing” plugs.

The H&E results of the “Bath” plug regularly produced levels of spermatozoa which would be considered, using the standard scale [16], “many in every field (++++)”. This recovery was not significantly affected by the presence or absence of body washes or dust/dirt particles. Although the ‘normal’ human spermatozoon is approximately 50–55 µm in size [18] it appears that 100 µm sized holes in the microfilter were sufficiently small enough to capture some of the spermatozoa as the bath water drained. One must assume that some of the spermatozoa became effectively trapped in the filter structure. In order to recover a greater quantity of spermatozoa it may be beneficial to reduce the size of the filter holes. However, the researchers found that the speed of water drainage was altered dramatically between normal drainage and when the plugs of the ERS were in place. The filter was slowing the drainage of water and one would predict that an even smaller-holed filter would increase this drainage time even further. This would be compounded when dust/dirt particles were added. This may not be practicable when the system is used ‘in the field’. The ability to recover spermatozoa should not be considered exclusively. The likelihood of the filter being blocked completely by larger particles of trace evidence such as hairs and fibres should also be taken into consideration. In most instances when dust/dirt particles were added, such things as fibres and hairs were easily visible on the surface of the microfilter. These results concur with initial research completed at Anglia Ruskin University [5] where the ability to obtain hairs, fibres and vegetation using the ERS was feasible. These particulates could simply be recovered via tweezers before the filter is subjected to spermatozoa extraction. As the filter is small, examination for hairs and fibres at this stage, using a low power microscope, is achievable and pertinent to complete at this stage.

There was a decrease in the number of spermatozoa observed when the “Rinsing” plugs were examined in comparison to the “Baths” plugs. There was also a decrease in the maximum amount of spermatozoa recovered at this stage. The H&E results were not affected by the presence or absence of body washes but there was a significant difference in recovery when dust/dirt was added. These results are particularly interesting as they show that some spermatozoa are retained on the walls of the bath and around the bath plug after the water has drained

from it during the “Bath” plug recovery stage. It also appears that the presence of dust/dirt particles played a positive role in capturing spermatozoa as they passed through the microfilter — probably by reducing the relative size of the filter holes and slowing the water drainage permitting greater transfer to the sides of the bath. The fact that recovery of spermatozoa is increased when dust/dirt is added is a particularly pleasing result as it implies that the ERS could be employed within a SARC to recover spermatozoa and other trace evidence types effectively. One would also urge Forensic Rescue Ltd. to consider a modified ERS system for scene examiners to use in the home of a victim who has showered/bathed post-sexual assault. The modified ERS could comprise a single microfilter plug for a general, thorough rinsing of the sides of the bath, followed by a wipe stage for any final trace material. As previously stated, this would need to be considered as an evidence collection tool which could be negatively impacted upon by extraneous DNA.

In conclusion, it is possible to obtain spermatozoa from bathwater through the use of the Evidence Recovery System. The authors suggest additional laboratory testing to elucidate whether these results can be replicated during a simulated shower. There was no effect on recovery in the presence of body washes but there was an increase when dust/dirt particles were added. These results indicate that the ERS could be deployed within a SARC setting as a non-invasive method to obtain forensic evidence from the victim of an assault who is unwilling to consent to a full medical examination. They also indicate the possibility of using a modified version of the ERS system to obtain spermatozoa directly from the bath or shower of a victim of a sexual assault who has already had a bathing experience after the incident. In both instances, further research is needed to elucidate the effect of the recovery stages on the ability to obtain a DNA profile, and the possible sources and likelihood of contamination from external source material.

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