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Verification of intimate and non-intimate recovery of DNA within Sexual Assault Referral Centres (SARCs)

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ABSTRACT

This paper describes the verification of DNA recovery processes undertaken in forensic medical examination facilities within Sexual Assault Referral Centres (SARCs) across England and Wales in the investigation of rape and sexual assault. This is in support of a national initiative for SARCs to provide additional quality assurances regarding forensic integrity. This is achieved through compliance with the Forensic Science Regulator (FSR) Code of Practice including accreditation to ISO 15189 *Medical Laboratories: Requirements for Quality & Competence*.

Existing national Faculty of Forensic & Legal Medicine (FFLM) recommended intimate and non-intimate DNA recovery processes were verified by five SARCs in a pilot study utilising both *in vivo* and *in vitro* testing. Three types of recovery scenarios were tested: 1) non-intimate recovery of touch DNA was undertaken from volunteers' skin following simulated struggles; 2) non-intimate recovery of blood, semen and saliva on simulated skin surfaces; 3) intimate recovery of known semen and saliva donors from gynaecological anatomical models. No contamination issues were observed in the non-intimate sample recovery exercises where the recovery technique is the same for live casework. However, with a minority of the intimate sample recoveries, some iatrogenic transfer of seeded DNA within the models was identified. Root cause analysis of the data led to the development of a new approach for training and known outcome competence assessment in intimate DNA recovery using gynaecological models seeded with invisible UV dyes to detect unintended transfer events. This verification exercise has led to the creation of the first SARC proficiency testing scheme.

1. Introduction

SARCs provide specialist medical services to victims of rape and sexual assault giving proactive support for the victim [1]. A key function of the SARC is to enable the victim to undergo a forensic and holistic medical examination to establish the health status of the person, record the injuries and to facilitate the recovery of forensic samples for evidential purposes [2]. While the recovery of forensic evidence is a vital function, the most imperative priority of a SARC is patient care, where their health and wellbeing are put first and where they will be treated with dignity and respect [3].

A number of high-profile miscarriages of justice involving forensic evidence have led to England and Wales seeking to quality assure all areas of forensic science and safeguard the integrity of the evidence presented to the Criminal Justice System (CJS). 'The Birmingham Six' is a high-profile case where wrongful convictions were made based on both confessions and inadequate forensic evidence in the 1970's [4]. Since February 2025, the University of Exeter Law School have identified 9 convicted sexual offence cases in England and Wales (between 1988 and 2013) that were later quashed due to false or misleading forensic evidence on their 'Miscarriages of Justice Registry' [5]. An example is the case of Christopher Scott P where the integrity of the DNA evidence was questioned by the CJS. He was convicted in 2002 for the rape of his ex-partner when anal swabs were adduced to prove anal intercourse had taken place, the conviction was overturned in 2004 when the possibility of cross contamination between the vaginal and

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anal swabs was presented [6]. The Home Office (a UK government department) completed a study in 2005, which indicated that a third of reported sexual offence cases are not progressed at the police stage due to evidential issues (21 % insufficient evidence; 13 % offender not identified; and 2 % no prospect of conviction) [7]. This has led to the creation of the independent Forensic Science Regulator (FSR) in 2007 [8] whose role is to ensure the reliability of forensic science delivered to the CJS in England and Wales. As part of the FSR's responsibility, the non-statutory Code of Practice was published determining requirements of forensic units to become accredited to ISO standards. On the April 29, 2021, the Forensic Science Regulator Act 2021 [9] received Royal Assent [10], requiring the FSR to publish a Code that defines the forensic science activities to which the Code applies, and the provision of statutory powers enacted in 2023. The published Code [11] includes specific requirements for the forensic medical examination of sexual offence complainants within SARCs. This directly coincides with the 2021 publication of the end-to-end rape review report findings and actions [12] and the prioritisation of the national strategy for prevention of Violence Against Women and Girls (VAWG) initiative. Quality issues have been identified within SARCs [12,13] and in response to these the FSR has mandated that all the facilities in England and Wales shall be compliant to the Code [11] and associated FSR guidance [14,15], which includes the requirement to meet the quality standard ISO 15189 *Medical Laboratories: Requirements for Quality & Competence* [16]. Part of these requirements is that processes used within SARCs including the recovery of forensic material for evidential purposes are validated. The recovery requirements followed by the SARCs are long established, detailed in the FFLM recommendations [17] which have been previously validated through scientific studies and publication in peer-reviewed journals and subject to bi-annual evidence-based reviews [18]. As per the FSR Code [11], following review of available validation data to determine if the validation is adequate, the SARC practitioners trained and signed off as competent in the method shall demonstrate that such adopted methods perform reliably at the given location by following the validated process [19,20]. This is a form of verification, and a key requirement of verification exercises is to include assessment of characterised material for which the expected outcomes are known [20]. For forensic medical examination facilities, recovery of touch DNA from skin-to-skin contact is relatively straightforward to experimentally verify and can be completed by, for example, volunteers simulating a struggle and then sampling from the 'victim's' wrist after it has been forcibly held by the 'perpetrator's' hand [21]. More challenging is the use of body fluids to verify recovery from intimate and non-intimate body areas, given:

a) potential ethical concerns,
b) health and safety risks,
c) practical constraints of identifying sufficient volunteers willing to be seeded with replicate sets of body fluids from a third party to then to be medically examined, and
d) use of live casework or post-coital volunteers, where the associated variations in individuals' anatomy means that any expected outcomes can never be assured.

For non-intimate sampling, these issues could be circumvented by using non-human skin alternatives. For example, pig cadavers have been used in the past to assess persistence of blood and touch DNA on the surface of skin submerged in water as an alternative to using human cadavers [22], but to date, little is documented on the use of alternative organic models. Recovery of body fluids from inorganic substrates is better characterised especially in studies of recovery optimisation [23] from a crime scene investigation perspective. *In vitro* studies lack the realism of using live tissue, including the absence of endogenous human DNA on the substrate surface. Mocked-up casework recovery from synthetic gynaecological models provides additional challenges in realistically mimicking *in vivo* surface texture, pliability and structure. A previous *in vitro* study (using methods not reflected by FFLM recommendations [17]) in which the external surfaces of vaginal models were seeded with a dye marker prior to swabbing internal vaginal surfaces identified trace dislocation of the marker to internal surfaces and concluded that *in vivo* studies were required to follow up this exercise [24].

This study verifies the processes for DNA recovery from victims of rape and sexual assault, by adopting a combination of *in vivo* and *in vitro* methodologies to assess both intimate and non-intimate recovery of target DNA. These were used to standardise materials and test procedures in a pilot exercise undertaken by five SARCs. This study provides the framework for all SARCs to use in their own verification exercises. The verification of intimate DNA recovery techniques highlighted a risk of unintended adverse movement of the seeded DNA, (iatrogenic transfer) within the gynaecological anatomical models during the sample recovery process. This may not be as prevalent during *in vivo* evidence collection due to intimate cavities of the model not replicating that of an individual. These data prompted the development of an innovative training approach that can be implemented nationally, using ultraviolet (UV) dyes to give real-time feedback, to forensic healthcare practitioners (FHPs), of potential points of contamination. Additionally, a method was developed to allow central standardised known donor seeding, national distribution and local recovery of the seeded substrates and anatomical models.

2. Materials and methods

Three SARCs participated in the first phase of this pilot in which both non-intimate touch DNA and biological fluids were recovered from non-intimate surfaces, plus intimate swabbing from the vagina and anus areas of anatomical models were undertaken. Lessons learned from this work were then applied to an updated exercise undertaken by a 4th and 5th SARC in phase 2 with the additional aim of assessing whether it is feasible for SARCs to conduct verification independently using remotely provided processes and test materials. Thirteen FHPs participated in total with either 2 or 3 representatives per SARC:

- SARC A: Phase 1: FHP 1 - 3
- SARC B: Phase 1: FHP 4–5
- SARC C: Phase 1: FHP 6–8
- SARC D: Phase 2: FHP 9–11
- SARC E: Phase 2: FHP 12-13

In all instances different mocked up casework scenarios were presented to the FHPs as part of an end-to-end process verification exercise within the context of which both non-intimate and intimate recovery of DNA samples were required; only the DNA recovery results of this wider exercise are described in this paper. Recovery was conducted using anti-contamination procedures in accordance with FFLM recommendations [17], employing the double swabbing technique [25,26] utilising forensic DNA grade Rayon Tipped Swabs (product code B22711-ETO) and forensic DNA grade water (product code B24511) supplied by SceneSafe™. All DNA recovery activities were conducted within the SARC forensic medical examination suites following procedures in line with the FSR Code [11] and FFLM recommendations [17] covering all relevant elements of the examination and forensic sample evidential recovery processes.

All the swabs were stored frozen and then submitted to the forensic service provider (FSP) (Cellmark Forensic Services) for analysis using their ISO:17025 accredited processes. The swabs were extracted and purified using the EZ1® and EZ2® DNA Investigator kit (Qiagen) and the BioRobot® EZ1xl workstation (Qiagen) and eluted into a 50 μL volume of Tris-EDTA (TE) buffer. Quantitation was performed using a 7500 Real-time PCR (RT PCR) system with Qiagen's Quantiplex PRO RT PCR kit. The quantification results were used to determine the volume of eluant and thus normalise the amount of DNA placed into the PCR

reaction. The samples were amplified using Thermo Fisher's AmpFLSTR™ NGM SElect™ PCR Amplification kit. Following amplification all samples were run on a 3500xl Genetic Analyser and the results were interpreted using Genemapper® IDX v1.5 software.

2.1. Non-intimate recovery of touch DNA

2.1.1. Phase 1

Three SARCs participated in phase 1 of this exercise and the experiment was repeated 8 times in total by 8 different FHPs, all using the same 2 volunteers (A & B).

Prior to DNA deposition, volunteer A ('perpetrator') washed their hands with soap, dried them on blue roll then vigorously rubbed them together. A moist and a dry control swab was then taken from their left hand. Volunteer B ('victim') washed and dried their hands as previously described, then moist and dry control swabs were taken from their left wrist. These controls were taken to monitor and evaluate background levels of DNA and only processed if contamination was detected. Volunteer A then created the touch deposition by using their right hand to forcibly grab volunteer B's (the 'victim') left wrist for approximately 20 s, applying both pressure and twisting friction akin to a struggle. Within an hour of this touch deposition occurring, an FHP sampled volunteer B's left wrist with a moist then dry swab, both from the topside and underside of the wrist. In addition, the FHP recovered a moist and a dry swab from the right wrist of volunteer B, as a control sample used for comparison as per FFLM guidance [17]. Reference buccal swabs were also taken from the participating FHPs and volunteers A and B.

2.1.2. Phase 2

Phase 2 was conducted after the phase 1 results were known. This was conducted at SARCs D and E for which detailed written instructions were provided rather than having on-site in-person guidance. The process was completed as per phase 1, except volunteers A and B were local SARC members of staff in which the 'perpetrator', after washing and drying their hands, then vigorously wiped their hands on their own face to maximise DNA transfer before continuing as before. Also, to minimise costs, controls were still taken but not processed unless a contaminant sufficient for comparison purposes was identified on analysis of the touch samples.

2.2. Non-intimate recovery of body fluid stains: blood, semen and saliva

Prior to conducting this pilot study, a pre-assessment was undertaken on the feasibility of using pig skin as an *in vitro* substitute for human skin. This testing identified practical limitations including difficulties in conducting effective DNA decontamination of the skin surface without causing damage, batch-to-batch inconsistency, and the logistics of using a substrate with a short shelf life that required refrigeration (results not shown). Subsequently textured vinyl was chosen as a substrate which has none of the aforementioned drawbacks but, in common with pig skin and other *in vitro* models, lacks endogenous human DNA.

Sections of textured vinyl upholstery fabric (Advanced Upholstery Service) were cut to size and decontaminated by duplicate spray/wipe cleaning cycles using 1 % Presept solution and blue roll, followed by a water spray/wipe. A target area measuring 3 × 3 cm for each of the stains were drawn on the surfaces which were then mounted in shallow storage trays and re-cleaned with a water spray/wipe.

Body fluids used in this experiment were diluted to improve their homogeneity and therefore the consistency of the amount of DNA in each replicate sample to be tested. Neat blood, semen and saliva samples were homogenised by vortexing with a whirlimix then diluted in Phosphate Buffered Saline (PBS) as follows: saliva 1 in 2, blood 1 in 10 and saliva 1 in 50, followed by further vortexing. For phase 1 experiments, 10 μL aliquots of each diluted body fluid were spotted in triplicate within the marked squares on the vinyl surfaces and allowed to dry a minimum of 2 h before being stored at −20°C until required. Prior to sampling, the trays were equilibrated to room temperature for a minimum of 2 h to remove condensation. For phase 2 experiments, the same approach was used but single sets of blood, semen and saliva deposits from different donors to phase 1 were used.

Individual sets of deposits (as shown in Image 1) were provided to each FHP participating in this pilot study. The FHPs within each SARC were presented with a different mocked up scenario (not detailed in this paper), the circumstances of which required recovery of the respective body fluid deposits from the victim's skin. Each sample was collected by moist and then dry swabbing as per FFLM recommendations [17] for non-intimate skin swabbing. A second section of unseeded vinyl was provided as a control surface to be sampled as required in the mocked-up scenarios.

The swab samples plus buccal swabs taken by FHPs were then frozen before being transferred for DNA analysis.

2.2.1. Non-intimate body fluids – proficiency testing exercise

Phase 3 was independent to the end-to-end verification study to further test the viability of the use of vinyl spotted with body fluids to be used as a proficiency test. This involved 10 vinyl surfaces seeded in the same manner as phases 1 and 2 with body fluid (saliva) which was prepared by the Forensic Information Database Services (FINDS). These were sent out to 11 different SARCs across the England and Wales and swabbed (using the moist and dry swab technique [17]) in two different areas on the vinyl representing the 'seeded' target area and an 'unseeded control' area.

2.3. Intimate recovery

2.3.1. Overview

Assessment of the intimate DNA recovery process was conducted using anatomical models which were seeded within the vaginal and anal areas to mimic a sexually assaulted adult female. The anal recovery process used here would equally apply to an adult male. Intimate forensic recovery ideally should be conducted without inadvertently introducing material potentially present on external surfaces of the model to the areas sampled internally. If this transfer from outer surfaces occurred in a real examination, this contamination could compromise interpretation and the reporting of an evaluative opinion by the forensic scientist for both source and activity level propositions. However, when evaluating findings and considering potential transfer of material from external areas to internal areas, the scientist should consider the levels of DNA material and its distribution across the areas sampled.

To assess this, body fluid from a second donor was applied to external surfaces of the model prior to the sampling process.

2.3.2. Model preparation

The anatomical models were Life/form® Advanced Pelvic Examination and Gynaecological Simulator SKU: LF01235 utilising Uterus "G" (LF01239), Pelvic organ block (LF01237) and "normal" genital pad (LF01238), all supplied by Nasco (images 2-5).

The models were disassembled and cleaned prior to each experimental setup; the pelvic organ block, uterus and genital pad were immersed in 3 % Presept solution for 15 min, scrubbed with a toothbrush, rinsed thoroughly with distilled water and left to dry on paper towels. Other surfaces of the model were decontaminated by spraying with Presept, wiping dry with paper towel, then repeating this spray/wipe process but with a sterile water spray.

Negative controls were taken by moist and dry swabbing prior to reassembling. The rectum was sampled first followed by the anal area then the endocervix and high vagina, followed by low vagina. The model was then reassembled, and swabs were taken from the vulval and perianal areas of the genital pad (shown in image 4).

In all but 2 instances, the models were seeded within the medical examination suites 1–3 h before examination, undertaken on-site by Forensic Capability Network (FCN) staff as technical support to the

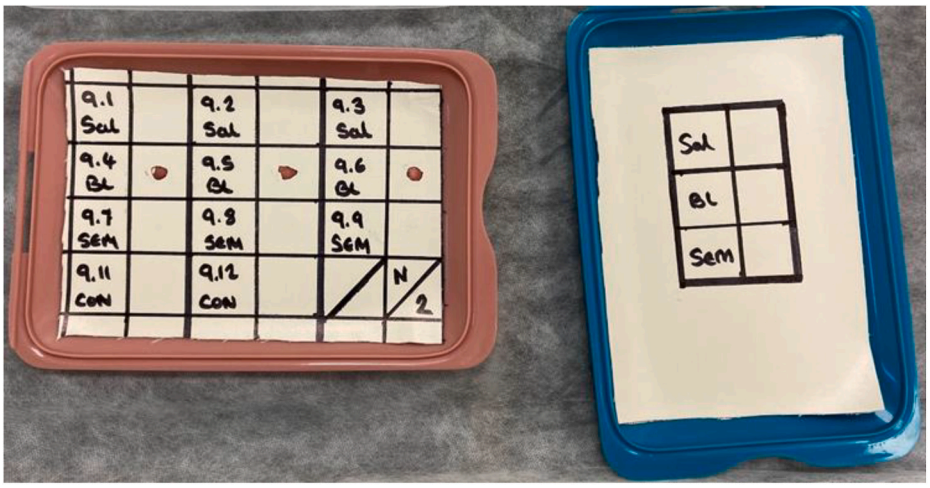


Image 1. Non-intimate swabbing - vinyl test surfaces.

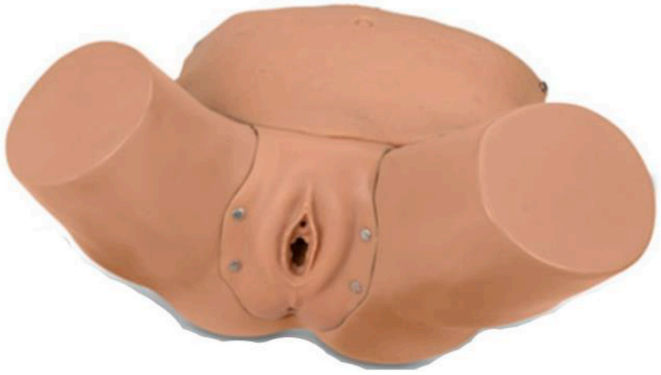


Image 2. Fully assembled model.

participating SARCs. The exception was two models used in phase 2 which were seeded remotely at FINDS (up to 4 days prior) then couriered to SARCs D and E where they were stored at ambient temperatures before being examined for DNA recovery. This variation was undertaken to assess the feasibility of remotely setting up and providing test models rather than undertaking this on-site immediately before examination. Semen and saliva samples from different donors were used, pre-mixed with equal amounts of forensic DNA grade Aquagel Lubricating Jelly (SceneSafe™) which was added to prevent the body fluids from running off the surfaces to which they were applied. The DNA profiles of these donors were compared to evaluate the number of shared alleles and this did not preclude being able to distinguish between the two individuals or affect the DNA analysis or interpretation.

For the first SARC that underwent this testing, a 1 in 50 dilution of semen in PBS was added to an equal volume of Aquagel due to contamination concerns about using very concentrated DNA test samples in a live SARC environment. However, these concerns were allayed as the study progressed and higher concentration semen samples were used in subsequent set-ups. Body fluids were applied to the models using swabs dipped into 200 µl of the mixtures pre-aliquoted in microfuge tubes. Two different set-ups were used: in 'set-up A', semen was applied first into the rectum facilitated by inserting a junior proctoscope into the anus, followed by temporarily removing the uterus to enable seeding of both the endocervix and high vaginal areas by entering from the back of the organ block, and finally saliva was applied to vulval and perianal areas. Care was taken to seed the external areas up to, but not into, the entrances of the anus and vagina as shown in image 5. In 'set-up B', the

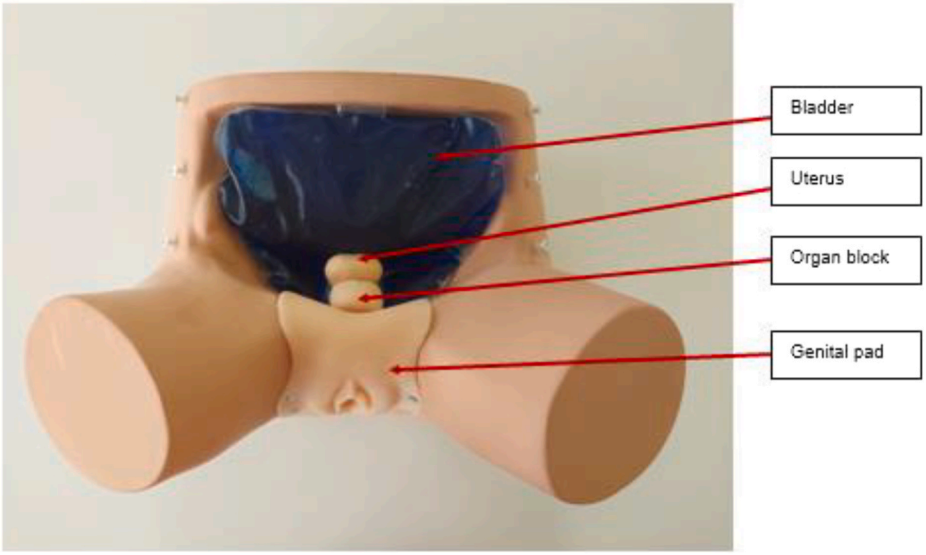


Image 3. Model with stomach cover removed.

Image 4. Genital pad.

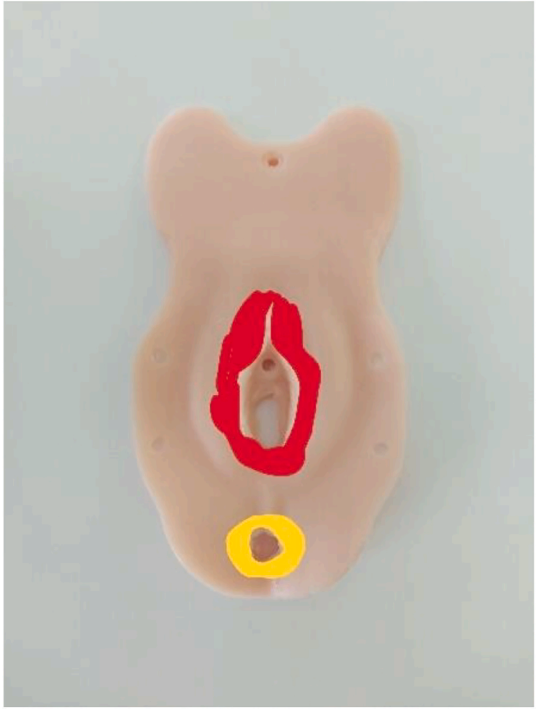


Image 5. Genital pad with vulval (red) and perianal (yellow) seeded areas illustrated.

body fluids were reversed with saliva applied internally and semen externally. It is acknowledged that the latter does not reflect a realistic real-life scenario, however it represents the most sensitive detectable arrangement to assess whether material may be introduced from external to internal surfaces, given that transfer of trace amounts of high DNA concentrated semen is less likely to be masked by the background saliva.

Each of the 11 FHPs participating in the pilot undertook a witnessed intimate recovery exercise as part of a simulated casework scenario, in which local examination procedures were followed, using forensic DNA grade consumables provided from the participating SARCs stores – (speculum, lubricating gel, swabs and water, all sourced from Scene-Safe™). Swabs were taken from the vulval and perianal area, low vaginal, high vaginal, endocervix, rectum and anus. Set-up B was assessed once at SARCs A, B, C & D, and set-up A either once or twice depending on the total number of participating FHPs.

Swabs taken from external surfaces seeded with saliva were processed by the FSP using an EZ1 extraction, whilst all others were processed using fast differential semen extraction enabling quantification values and DNA profiles including allele count numbers to be determined for both the epithelial and seminal fractions of the extracts.

2.3.3. Training & competency assessment

2.3.3.1. Training & competency assessment: set up. A couch cover was placed over the examination couch and the gynaecological model was placed on top, where it was seeded *in situ* at the examination site. The dummy was dismantled then seeded using a combination of 3 different coloured invisible UV body paints as per the seeding schedule (Table 1).

The gynaecological model was then reassembled, each section was checked with the UV light to ensure the correct areas have been seeded with the allocated colour and that there has been no transfer of the dyes to any areas other than those specified in Table 1.

2.3.3.2. Training & competency assessment: examination. This training consisted of a background presentation (on FSR regulations and FFLM guidance) followed by a practical demonstration of set up of and recovery from the anatomical model. The training was delivered by a Forensic Quality Specialist and a highly experienced FHP who was a Faculty Officer of the FFLM.

Over the training and competency assessments, 65 seeded anatomical dummies were set up by 6 different operators, following training, using local anti-contamination procedures in accordance with FFLM guidance [17] these were examined by 65 FHPs based on their interpretation of the following mock case scenario:

'Patient has presented at the SARC following a vaginal and anal rape'.

Under observation by the trainer, the trainee FHP recovered swabs as per FFLM guidance [17]. These swabs were assessed under UV light to determine whether the correct areas have been sampled based on the Training & Competency Seeding Schedule (Table 1).

The acceptance criteria for this assessment are shown in Appendix A.

3. Results

3.1. Non-intimate recovery of touch DNA

Table 2 provides an overview of the results from the non-intimate touch DNA samples recovered and graph 1 shows the allele count of each of the swabs recovered following the skin-to-skin contact. It was expected that the samples recovered from the 'victim' will match either the 'victim', the 'perpetrator' or both, their full profiles (total 32 alleles) are not expected to be recovered.

Overall, 19 of the 21 skin swabs taken from the wrist of the 'victim'

Table 1
Training and competency seeding schedule.

Order of seeding	Gel Colour	Location
1	UV Gel colour A (Blue)	Endocervix
2	UV Gel colour A (Blue)	High vagina
3	UV Gel colour B (Orange)	Rectum
4	UV Gel Colour C (Green)	Perianal
5	UV Gel Colour C (Green)	Vulva

Table 2
DNA profile detection acceptance criteria and number of FHPs who met each and average DNA profile allele results from the swabs recovered following skin-to-skin contact.

Phase		Phase 1			Phase 2	
SARC		SARC A	SARC B	SARC C	SARC D	SARC E
Acceptance criteria per FHP	Only Perpetrator DNA recovered	0	0	1	0	1
	Only Victim DNA recovered	2	1	0	0	0
	Perpetrator & Victim DNA recovered	0	0	1	3	1
	Perpetrator & Victim DNA and additional DNA insufficient for comparison recovered	0	0	0	3	0
	Victim DNA and additional DNA recovered insufficient for comparison	3	2	4	0	1
	Total DNA Insufficient for comparison	1	0	1	0	0
Allele count results	Average Allele Count Per Phase	17			25	
	Average Allele Count Per SARC	15	14	24	34	10
	Allele Count Range Per SARC	19	19	37	12	2

Incomplete DNA Short Tandem Repeat (STR) profiles with very little genetic information have been determined as insufficient for comparison to known DNA profiles.

Graph 1. Bar graph to show DNA profile results from swabs recovered following skin to skin contact. Orange represents the samples recovered from the left wrist underside. Blue represents the samples recovered from the left wrist top side.

(post mock struggle) yielded profiles matching either the victim alone, the perpetrator alone or a mixture of the perpetrator and victim. 2 samples (T3 and T14) yielded results insufficient for comparison purposes, 2 matched only the perpetrator and 3 matching both the victim and the perpetrator with additional DNA identified that was insufficient for comparison. No statistically significant difference in amount of DNA recovered was observed between the upper and lower wrist swabs (*t*-test results shown in Appendix B.1). In all phase 1 and 2 results, all alleles observed that were additional to the aforementioned matches were assessed to be insufficient for comparison purposes, therefore, no contamination was attributable to any of the FHPs who recovered the samples.

The average allele count in phase 1 was 17 and 25 in phase 2; 1 out of the 16 samples (6.25 %) in phase 1 yielded the perpetrator's DNA and 4 out of the 5 samples (80 %) in phase 2. The recovered DNA was significantly higher in phase 2 compared to phase 1 (*t*-test results shown in Appendix B.2). Overall, 23.8 % of the 'victim' wrist samples recovered post contact with the 'perpetrator' identified the 'perpetrator's' DNA.

An Analysis of Variance (ANOVA), shown in Appendix C, determined that the differences between the averages of alleles recovered from the victims' wrists, when compared between SARCs, is not big enough to be statistically significant.

3.2. Non-intimate recovery of body fluid stains

The expectation for this experimentation was that the FHP would recover sufficient DNA from the seeded deposits of body fluid on the vinyl to match the DNA of the body fluid donor. There should be no DNA contamination that is sufficient for comparison and all control samples should not recover sufficient DNA for a comparison.

In all instances, the expected DNA profiles were obtained from the swabs taken from the 87 body fluid stains in both phase 1 and phase 2. Allele count results and quant scores are shown in Table 3 and average results shown in graph 2. Alleles in addition to the donors' profile were observed in 13 samples. The potential of this being attributed to operator contamination was investigated, 43 % of these additional allele markers are consistent with the operators' DNA, however this was not conclusive, as the DNA was insufficient for comparison purposes. The majority of these samples displaying additional alleles, 9/13 (69 %), were identified in the semen samples amounting to 31 % of the total semen samples displaying additional alleles.

The overall pattern of results was the same with each SARC in both phase 1 and 2 achieving highest recovery from the semen samples as shown in graph 3 which also demonstrates the distribution of these results and the lack of outliers within these data.

All 13 control samples recovered 0 alleles, with the exception of one, where 2 alleles were recovered, insufficient for a comparison.

There were minimal differences observed in average yields between SARCs in both phase 1 and 2 for the same body fluid types, but these were not significantly different. All results from this testing are shown in Table 3. **Note:** Phase 1 and phase 2 results were generated from different sets of donor samples.

3.2.1. Non-intimate body fluids – proficiency testing exercise

Results from this proficiency testing exercise are shown in Table 4 where all 11 FHP participants met the test expectation, recovering sufficient DNA matching the body fluid donor, without any contaminating DNA sufficient for comparison. The test expectation for the control samples were met by 10 of the 11 FHPs, with one FHP producing a profile for the negative sample matching that of the body fluid donor.

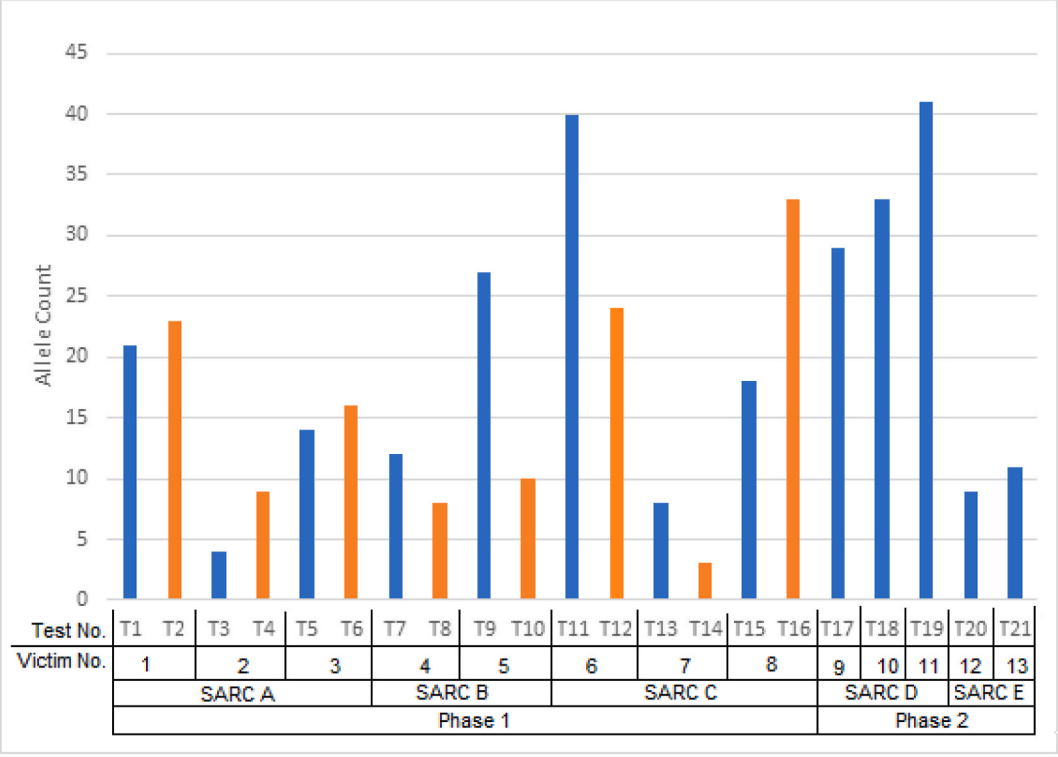
3.2.2. Intimate recovery of body fluids from the vagina and rectum

The acceptance criteria for the intimate recovery experiments are shown in Table 5.

The results obtained from all 11 participating FHPs are summarised in Table 6 below and detailed in appendix D.

Table 3
Allele count and quant score results for each body fluid recovered per SARC.

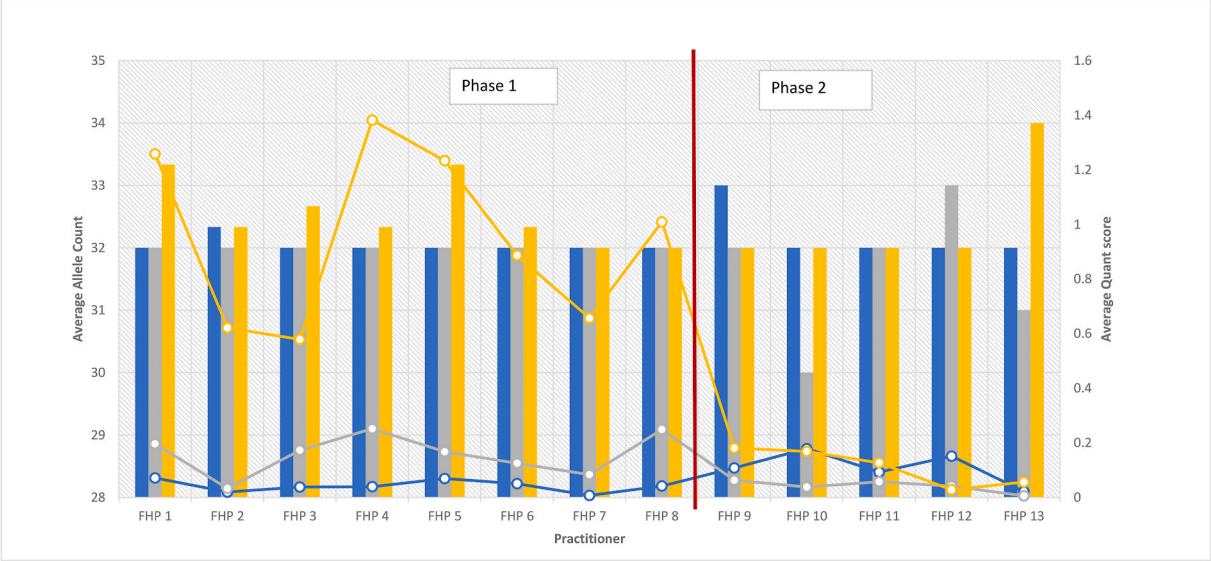
Sample Information			Allele Count			Quant Scores		
SARC	Phase	Sample Number	Saliva	Blood	Semen	Saliva	Blood	Semen
1	**1**	FHP 1	32	32	32	0.1566	0.0474	0.9246
			32	32	32	0.1341	0.0738	1.3509
			32	32	36	0.2983	0.0932	1.4983
		FHP 2	32	33	32	0.0256	0.0036	1.0637
			32	32	33	0.0455	0.0148	0.186
			32	32	32	0.0286	0.0409	0.6141
		FHP 3	32	32	33	0.1592	0.0314	0.301
			32	32	32	0.1901	0.0255	0.8726
			32	32	33	0.1693	0.0575	0.5623
2		FHP 4	32	32	32	0.0817	0.0412	1.4794
			32	32	33	0.5581	0.0399	1.3601
			32	32	32	0.1157	0.0371	1.3054
		FHP 5	32	32	33	0.1845	0.0969	1.3474
			32	32	32	0.1671	0.0725	1.1912
			32	32	35	0.151	0.0384	1.1614
3		FHP 6	32	32	32	0.1384	0.0681	1.1649
			32	32	32	0.1588	0.0406	0.7181
			32	32	33	0.0785	0.0424	0.7761
		FHP 7	32	33	32	0.1095	0.0033	0.5258
			32	31	32	0.1206	0.0029	0.8365
			32	32	32	0.0204	0.0163	0.6075
		FHP 8	32	32	32	0.2284	0.0458	0.8408
			32	32	32	0.2812	0.0359	0.9883
			32	32	32	0.2372	0.0442	1.1986
4	**2**	FHP 9	32	33	32	0.0633	0.1081	0.1808
		FHP 10	30	32	32	0.0381	0.1788	0.1686
		FHP 11	32	32	32	0.0575	0.093	0.1266
5		FHP 12	33	32	32	0.0416	0.1512	0.02825
		FHP 13	31	32	34	0.0059	0.0217	0.0562



Graph 2. Non-Intimate swabbing DNA results. Blue = Allele count - Blood; Blue line = Quant score - Blood; Grey = Allele count - saliva; Grey line = Quant score - Saliva; Yellow = Allele count - Semen; Yellow line = Quant score - semen.

3.2.3. External seeded surfaces: vulval and perianal

The perianal and vulval surfaces were seeded with either saliva using set-up A, or semen using set-up B. In 21 out of 22 results only the correct DNA profiles corresponding to the seeded body fluids were generated from the swabs taken, and where additional alleles were observed these were insufficient for comparison purposes. Therefore, all these results met the acceptance criteria. The one result that failed to meet the acceptance criteria was from the vulval swabs taken by FHP1 which



Graph 3. Box & whisker: Body fluid results per FHP based on quant scores (µg/l).

Table 4
Proficiency Testing exercise results.

SARC No.	Saliva Sample			Control Sample		
	Allele Count	Match to the donor	Any additional DNA sufficient for profiling	Allele Count	Match to the saliva donor	Any additional DNA sufficient for profiling
SARC 1	34/34	Yes	No	0/34	No	No
SARC 2	34/34	Yes	No	0/34	No	No
SARC 3	34/34	Yes	No	0/34	No	No
SARC 4	34/34	Yes	No	0/34	No	No
SARC 5	34/34	Yes	No	0/34	No	No
SARC 6	34/34	Yes	No	0/34	No	No
SARC 7	34/34	Yes	No	0/34	No	No
SARC 8	34/34	Yes	No	0/34	No	No
SARC 9	34/34	Yes	No	16/34	Yes	None in addition to the saliva donor
SARC 10	34/34	Yes	No	0/34	No	No
SARC 11	34/34	Yes	No	0/34	No	No

detected both the correct donor (saliva) DNA plus a further contributor (s) suitable for comparison, with no matches observed against FCN or FSP staff.

3.2.4. *Internal unseeded surfaces: low vagina and anus*

The low vagina and anus were not targeted for seeding in this exercise. In total, 21 of the 22 results from these regions met the acceptance criteria, for which presence of either internally or externally seeded DNA was acceptable but not DNA from another source apart from the FCN operator setting up the experiment. In combination, 11 out of the 22 results from these areas gave zero or insufficient DNA for comparison purposes; 8 identified the DNA used to seed the internal surfaces

Table 5
Acceptance criteria for samples recovered in the intimate swabbing exercise.

Swabbing Area		Acceptance Criteria for Swabs Recovered
A	Vulval and Perianal	Only the DNA seeded onto the external vulval and perianal surfaces recovered, with no other DNA present that is sufficient for comparison.
B	High Vaginal and Endocervix	Only the DNA seeded onto the internal high vaginal and endocervical surfaces recovered, with no other DNA present that is sufficient for comparison.
C	Low Vaginal & Anal Canal	Only DNA seeded onto the internal or external surfaces is recovered, with no other DNA present that is sufficient for comparison.
D	Rectum	Only the DNA seeded onto the internal rectal surface, with no other DNA present that is sufficient for comparison.
E	All	Sufficient DNA to give a searchable profile matching the deposited body fluid is recovered from the seeded areas of the model.
F	All	Additional alleles insufficient for comparison observed additional to the expected profile

Note: Detection of a non-donor DNA (contaminant) sufficient for comparison purposes results in a failure to meet the above criteria. The exception to this rule is if the contamination is a direct match to the operator setting up the experiment as this constitutes a shortcoming in the experimental set-up and not a failure in verification of the sampling process conducted by the SARC.

(i.e. high vaginal, endocervix and rectum) with or without detecting additional alleles insufficient for comparison purposes; FHP 6 low vaginal swabs detected both internally seeded DNA (semen) plus a lower level of externally applied saliva DNA; and external seeding DNA alone or this plus DNA alleles; FHP 2 anal swabs detected both DNA from internally applied semen and a partial profile from the operator responsible for cleaning and set up of the models for the recovery experiments. The single failed result was from FHP 1 low vaginal swabs which recovered DNA from one or more unsourced individual(s) with no matches observed against FCN and FSP staff.

3.2.5. *Internal seeded surfaces: endocervix, high vagina and rectum*

In total 11/11 results from swabs of the seeded endocervix yielded acceptable results with the expected DNA profiles detected according to the body fluids used for seeding. These results either contained no additional alleles or those present were insufficient for comparison purposes.

In total 6/11 results from high vaginal swabs met acceptance criteria

Table 6
Recovery of DNA from anatomical models - number of alleles identified from 7 sampled regions.

Verification Phase	SARC	Experimental Set-Up	FHP	(External) Vulva	(Internal) Low Vaginal	(Internal) High Vaginal	(Internal) Endocervix	(External) Perianal	(Internal) Anal	(Internal) Rectum
PHASE 1	A	A	FHP1	41	30/0	11/32	34/32	38	35/32	13/21
		B	FHP2	27/30	30/32	9/29	32/4	2/32.	33/18	33/2
		A	FHP3	34	4/0	32/32	34/37	34	11/0	7/0
	B	A	FHP4	33	0/0	33/33	32/33	32	32/34	33/32
		B	FHP5	32/32	1/0	32/1.	32/4	32/32	19/21	21/4.
	C	A	FHP6	32	47/32	30/33	32/33	33	34/32	32/32
		B	FHP7	33/32	0/2	7/30	32/0	33/32	34/1	36/9
		B	FHP7	n/a	n/a	4/7.	n/a	n/a	n/a	32/1
		A	FHP8	32	32/33	34/33	33/32	34	35/33	33/33
PHASE 2	D	A	FHP9	32	0/0	3/5.	14/14	0/32	0/0	13/5.
		B	FHP10	17/31	3/1.	32/0	32/0	27/33	3/5.	32/0
		A	FHP11	32	0/0	0/0	30/31	34	4/6.	36/10
Overall first pass rate				10/11.	10/11.	6/11.	11/11.	11/11.	11/11.	7/11.

Notes.
• x/y is number of alleles from the epithelial/seminal fractions respectively where differential extraction is performed. Note: the differential extraction process is not an absolute separation of the epithelial and seminal components, so for example the seminal fraction may still contain epithelial DNA e.g., from saliva if present in a semen/saliva mixture.
• Numbers in red do not meet experimental acceptance criteria as detailed in Table 5.

in which the seeded DNA profile was determined with or without additional alleles that were insufficient for comparison purposes. Of the results that failed, FHP 9 and FHP 11 yielded insufficient alleles for a comparison to be made; FHP 2, 3 and 7 detected both the internally seeded profile plus sufficient additional alleles to positively match the externally seeded DNA. FHP 7 was subsequently repeated with a newly set-up model and yielded a match to the internally seeded DNA plus a low-level profile was also detected sufficient for comparison purposes that was unsourced.

In total 7/11 results from rectal swabs met acceptance criteria in which the seeded DNA profile was determined with or without additional alleles that were insufficient for comparison purposes. Of the results that failed, FHP 3 did not detect the seeded DNA but generated a partial profile from an unsourced contaminant; FHP 1, FHP 7, and FHP 11 detected both the internally seeded DNA plus partial profile contaminants matching the externally seeded DNA. FHP 7 was subsequently repeated with a newly set-up model and yielded a match solely to the internally seeded DNA. Overall, proportionately more external to internal transfers were observed with semen seeded onto the external surfaces compared with saliva, but the sample size is too low to draw a definitive conclusion from this observation. In routine SARC casework, any saliva recovered on an internal swab is unlikely to be detectable as the cellular DNA would be swamped by the high levels of donor vaginal DNA.

3.2.6. Control results

Where profiles additional to those expected were generated, these were compared against participating FHP and FCN staff profiles created for this exercise plus against the FSPs Staff Elimination Database (SED). No matches were observed against any FHPs or FSP staff, but 2 contamination instances were attributable to the FCN member of staff responsible for setting up all the recovery exercises: partial profiles matched in FHP 2 anal swab result, and the negative control swab taken from the anal area for FHP 8. Unfortunately, FHP 1 declined to provide an elimination sample so they cannot be eliminated as a source of the contaminants observed in 3 of their sample recoveries.

The negative controls for the anatomical models were sampled directly after cleaning and before being deployed to the SARCs for body fluid seeding. The one exception was the model sampled by FHP 9 which was seeded before it entered the SARC. Therefore, any contamination observed in these samples could not have occurred within the SARCs. Negative control results are detailed in appendix D. In total, 56 negative controls were taken and processed from the models examined at SARCs A, B and C. Controls were also taken at SARC D but were not processed as no contamination from the recovered samples required investigation. Of the controls, 46/56 yielded either zero detectable contamination or insufficient for comparison purposes; the negative control swab taken from the anal area for FHP 8 yielded a partial profile that matched the FCN member of staff responsible for setting up all the recovery exercises; the remaining 9 controls yielded partial profiles sufficient for comparison purposes but were unsourced in comparison against FHP, FCN and FSP staff, 7 of these unsourced contaminants were from the assessment of SARC A and none were detected in the examination swabs taken by the FHPs.

3.3. Intimate swabbing training & competency assessment

Three national training events were held across England and Wales where 30 FHPs from different SARCs participated, each of these FHPs rolled out this training assessment to FHPs within their organisation, not all results are included in this paper. In total 34 out of 65 trainee FHPs did not meet the acceptance criteria (shown in Appendix A: Table 7) for at least one swab. Out of these 34 FHPs, 77 % did not meet the acceptance criteria on the rectal swabs they recovered as they were observed to have included transfer of UV gel from the perianal and/or anal canal to the shaft or tip of the rectal swab; 17 % had transfer of the UV gel from the vulval area or vaginal opening onto the shaft or tip of the high vaginal swab. The remaining 6 % found the above transfer on both the rectum and high vaginal swabs recovered. In all instances, following discussion with the trainer and a greater focus on the positioning of the speculum or proctoscope, ensuring the swab does not make contact with the consumable, resulted in the FHP successfully meeting all elements of the acceptance criteria. To note; the collection method used *in vivo* is slightly different to that used in the anatomical models where during this exercise the FHP's had to tilt the speculum/proctoscope and swabs to enable sample collection which is not required in *vivo* sample collection.

4. Discussion

4.1. Non-intimate recovery of touch DNA

In the recovery exercises following non-intimate skin to skin contact, sufficient DNA material was recovered from the 'victim' matching the 'victim' and/or 'perpetrator' from all but 2 of the 21 paired moist and dry swabs taken (91.5 %). Overall, 23.8 % 'victim' swabs recovered post struggle identified the perpetrator's DNA. The 2 samples that did not recover sufficient DNA for comparison does not indicate poor sample

technique, the purpose of the non-intimate DNA recovery is to recover suspect DNA and ideally none/low level victim DNA as it could swamp the suspect DNA during the DNA analysis and interpretation process.

No DNA contamination from the participating FHPs was detected and this enabled the recovery process to be verified at all 5 SARCs. Differences in how readily individuals transfer DNA from their skin is well documented [21,27,28] and this variability introduces uncertainty into conducting the verification exercise when using volunteers whose shedder status has not been previously determined. However, the chances of detecting successful transfer can be improved by increasing levels of DNA on the skin contact area of the 'perpetrator' prior to the simulated struggle as per the modified process applied at SARC D and E in phase 2 which resulted in confirmed match detection of the perpetrator from 6.25 % in phase 1, to 80 % in phase 2. An ANOVA, shown in Appendix C, determined that there is no statistical difference in results between SARCs.

4.2. Non-intimate recovery of body fluid stains

Verification of non-intimate recovery of blood, semen and saliva stains did not present issues at any of the SARCs; no contamination was detectable from any of the FHPs and in all instances the DNA profiles from all the 87 recovered body fluids were as expected, matching the donors DNA, with the majority of samples providing full profile matches. Where additional alleles where detected these were not sufficient for comparison. It is acknowledged that this *in vitro* study lacks the realism of using live tissue, including the absence of endogenous human DNA on the substrate surface, but it did enable successful recovery to be demonstrated in all instances without introduction of contamination. Vinyl does have significant advantages in enabling essentially identical multiple replicate test sample sets to be generated, which in turn could enable potential differences in individual practitioner performance to be assessed if sufficient numbers of replicates were to be processed. Differences in performance between SARCs in average recovery yields were seen but did not identify statistically significant differences, indicating that site-to-site differences in applying the FFLM recommended recovery processes [17] were also not significant. Use of test sets of body fluids on vinyl surfaces also effectively circumvented potential ethical, health and safety and participant concerns of applying blood, semen and saliva from a third party on to a volunteer's skin. The seeded vinyl test surfaces can also be readily stored frozen which aids in the logistics of conducting this type of exercise.

4.2.1. Non-intimate body fluids – proficiency testing exercise

The success of the non-intimate recovery of body fluids technique using vinyl surfaces seeded with body fluids led to the development of the first UK SARC proficiency test. All 11 SARCs who participated, recovered the donors' DNA without contamination and no contamination from the control samples with the exception of 1, where the donor profile had been recovered from the control sample. Upon further investigation, this may have been due to an error where the FHP had swabbed the wrong area for the control sample. These results demonstrate the suitability of this technique as a PT to be utilised to test the competency of FHPs and compare SARCs performance which before now has never been explored. FINDS have now set this up as a permanent PT Scheme available nationally.

4.3. Intimate DNA recovery

All samples recovered at SARC B were without issue, and met the acceptance criteria in full, i.e. sufficiency of recovery, giving the expected DNA profiles and without contamination of any significance. At each of the other 3 SARCs there was evidence of transfer from the external perianal and/or vulval surfaces to some of the recovered rectal and high vaginal samples, respectively. The root cause of this transfer was investigated through the development of a dye-marking process intended for training and competency assessment. This demonstrated an obvious 'snow-plough' effect at the edges of an inserted proctoscope (shown in Image 6) indicating that the observed contamination events may be caused by a combination of the non-lifelike characteristics of the anatomical model combined with the sampling technique of the FHPs.

This was investigated further through the training and competency assessment, results of this are described below. The fact that approximately half the participating FHPs did not encounter this transfer issue suggests that slight differences in individual technique could be a contributing factor. It would be worthwhile looking in detail at the approach used at SARC B as an example of best practice, given that this was the only facility without any adverse DNA results. That said, all 11 FHPs were successful in recovering DNA from the endocervical region without any contamination issues. One approach to assessing the real-life risk of transfer would be to seed live volunteers on external surfaces with the dye markers, then conduct internal examinations and determine whether the risk of external to internal transfer is being effectively managed by existing processes operated by trained and competent staff.

Further work could be conducted on the test results where contamination from external areas has been identified on internal samples to quantify the level of transfer, identifying how many alleles were introduced from the external seeding as this is pertinent information for FSPs when they are investigating SARC casework when addressing a penetrative act and determining its significance to the case.

In UK FSPs, forensic scientists interpreting and evaluating the findings from SARC evidence in relation to any intimate swabs, will consider the levels and distribution of sperm cells and/or DNA across the set(s) of swabs examined. However, the significance of the findings will be case specific and dependent on the case information provided by both the prosecution and defence, and will consider the time since the incident, any factors that may impact on the persistence of semen following the incident, together with other findings in relation to the case, such as the presence of blood and the Acid Phosphatase (AP) or Prostate Specific Antigen (PSA) reactions obtained from the swabs, together with the findings in relation to clothing and other pertinent exhibits. Therefore, even if similar examination results are obtained in two different cases, the interpretation and conclusions may be different.

The single failed results within both the vulval and low vaginal swabs across the 4 SARCs was from FHP 1 which could be attributed to swab holding technique. During the exercise, it was observed that the practitioner held the swab by the shaft rather than using the end of the swab – a technique that goes against best practice methods. This further highlighted the benefits that the developed training and competency approach from this exercise can add, despite its limitations of 'realism' and differences to a human body. The DNA source of these failures could not be fully investigated as FHP 1 was not willing to supply a reference sample for direct comparison.

With regard to contamination observed in negative controls, SARC A was the first to undertake the pilot exercise and displayed significantly higher levels of background contaminating DNA compared with the subsequently tested SARCs. This indicates improvement through increasing experience in undertaking the cleaning, swabbing and seeding activities in this verification exercise which has no precedent. The models used at SARC A were prepared in a clean room facility, whilst subsequent set-ups which gave cleaner background levels were performed in less ideal surroundings, indicating the detail of the cleaning process is more impactful than the surroundings within which it is conducted.

More tests are warranted to confirm the feasibility of remotely setting up the seeded anatomical dummies and couriering these to the point of testing rather than undertaking set-up locally. Only 1 of the 11 models, used by FHP 9 at SARC D, was remotely prepared in this way but gave results comparable to the other models tested at the same SARC. This has been combined with remote set-up and supply of the body fluid-seeded vinyl test surfaces, together with detailed instructions for SARC

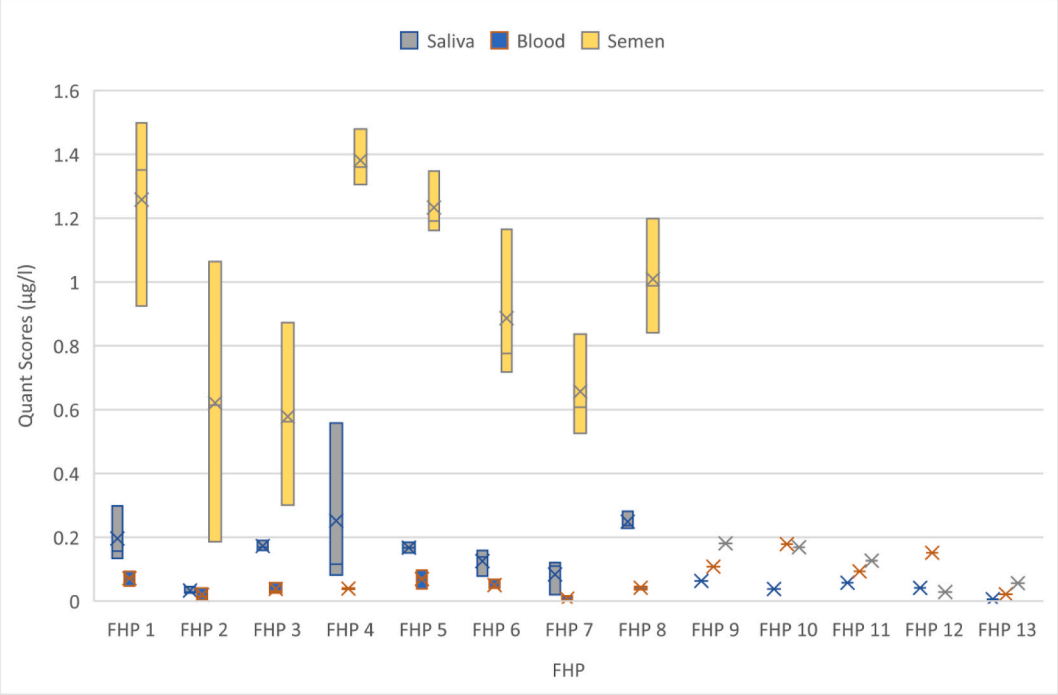


Image 6. Photograph of 'snow plough' effect seen during UV dye training exercises. The yellow dye was seeded at the entrance of the anus.

personnel to conduct their own skin to skin sampling experiments, which together provide a comprehensive and cost-effective approach to facilitate other SARCs in verifying their DNA recovery processes.

4.4. *Intimate swabbing training & competency assessment*

It is recognised that the restricted pliability of the orifices of gynaecological models increased the technical difficulty, specifically of the rectum and high vaginal recovery, compared to *in vivo*. For example, *in vivo*, the vagina and rectum would be collapsed, and sampling of the mucosa beyond the tip of the proctoscope or speculum may be technically simpler (provided the examinee remains still).

Lubricant jelly was used to reduce friction and create a barrier when inserting the speculum and proctoscope as per national recommended processes [17]. This did not completely eradicate iatrogenic transfer as it was observed on the edges of both the speculum and the proctoscope. Iatrogenic transfer onto the shaft or tip of the swab most commonly appeared to be due to contact with either the proctoscope or speculum. It was possible to eradicate transfer to the shaft or tip of the swab when the FHP repeated the process focussing on avoiding contact of the swab with the proctoscope or speculum. This appeared to be reproducible with DNA as discussed above.

Despite the limitations of the gynaecological model compared to *in vivo* collection, the principles to increase forensic integrity and awareness of potential contamination risks are transferable.

An incidental observation when completing this exercise was the risk of lubricant drainage from the vulval area to the perianal area under the influence of gravity. This is likely also a risk during *in vivo* sampling and was raised with the FFLM Intercollegiate Forensic Science Subcommittee (FSSC) and resulted in the publication of recommendations on the order of ano-genital sampling when obtaining forensic specimens from complainant and suspects of sexual offences [29]. This training approach has been implemented in over 40 SARCs across England and Wales and can be utilised alongside other methods for example, live casework data, observation, additional proficiency testing on other aspects of the examination (e.g. triage) to form a holistic training and competency framework.

The low incidence of contamination provides support that the measures to minimise contamination were effective in the SARCs undertaking the study. The contamination incidences observed and investigated reinforced the FSR requirements for staff involved in the recovery of body fluids for DNA analysis to be included on an elimination database [11]. In this study and for proficiency test providers that would also include those setting up any test materials.

5. Conclusions

This paper describes the verification of intimate and non-intimate DNA recovery processes in line with FFLM guidance [17], conducted within five SARCs in England and Wales that meets compliance with the FSR Code [11] and ISO 15189 [16]. This study provides a template that other SARCs can utilise in their own verification exercises.

Follow up work is required to improve data collection regarding the incidence and prevalence of iatrogenic transfer during examination to quantify more clearly the risk posed to the CJS and further evidence that existing processes and mitigations are effective. This could be achieved through further analysis of existing data from this research and live case work to quantify the iatrogenic transfer identified. This study has resulted in the development of the first SARC intimate and non-intimate DNA recovery proficiency test which is now available from FINDS in the UK.

The training technique of dosing gynaecological models with UV dye has provided an innovative approach to understanding forensic integrity risks and improving FHP intimate swab recovery techniques. It gives real-time visual feedback and allows consistent implementation across a national platform. This meets the requirement within the FSR Code [11] of both initial and ongoing known outcome competency assessments for intimate swabbing, which is a more cost-effective alternative to using known DNA sources. This method provides a viable alternative to *in vivo* training challenges with ethical, health and safety and practicalities which has now been adopted by the majority of SARCs across the UK. Follow-up work is required, ideally by means of *in vivo* studies, to demonstrate that the real -life risk of external to internal transfer resulting from the examination process is being effectively managed.

This study demonstrated the reproducibility of DNA transfer with the use of UV dyes as a real time, more cost effective alternative. With further experimentation this technique might prove to be a suitable approach for DNA laboratory or Crime Scene Investigation (CSI) verification or training and competency assessments.

CRediT authorship contribution statement

Michelle Gaskell: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **June Guiness:** Writing – review & editing. **Amy Hamm:** Writing – review & editing, Validation, Methodology. **Guylaine O. Hanford:** Writing – review & editing, Visualization, Resources, Project administration, Investigation. **Abi Marshall:** Writing – review & editing, Methodology. **Kevin Sullivan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval

No SARC patients were involved in this study. Body-fluid donors and participating SARC personnel provided written consent prior to involvement.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Abi Marshall reports a relationship with Mountain Healthcare that includes: employment. Amy Hamm reports a relationship with Partnering Health Limited that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

Appendix A Table 7
Intimate swabbing training & competency assessment results acceptance criteria

No.	Acceptability	Criteria
1	The correct swabs have been recovered based on the scenario.	The following swabs are recovered at minimum: these are at minimum: - Vulval (moist & dry) - Low Vagina (dry & dry) - High Vagina (dry & dry) - Endocervical (dry & dry) - Perianal (moist & dry) - Anal (dry & dry) - Rectum (dry & dry)
	Samples are recovered as per the FFLM Guidelines.	FFLM Guidelines: Recommendations for the collection of forensic specimens from complainants and suspects [1] has been followed
2	Correct glove changes have taken place	Gloves to be changed as per the SARC SOP. At minimum outer gloves are replaced between vaginal and anal examinations. No dye on the gloves post examination.
3	Correct PPE worn	PPE worn as per FFLM Guidelines: Recommended Equipment for obtaining forensic samples from complainants and suspects [2] and as per SARC procedure
4	Correct consumables used	Consumables used as per FFLM Guidelines: Recommended Equipment for obtaining forensic samples from complainants and suspects [2] and as per SARC procedure
5	Correct UV gel recovered from the vulva	Only colour A recovered from vulval swabs
6	Samples packaged and labelled correctly	All samples packaged and labelled as per FFLM Guidelines: Recommendations – Labelling Forensic Samples and as per SARC SOP.
7	Correct UV gel recovered from the high vagina	Only colour A recovered from the high vaginal swabs
8	Correct UV gel recovered from the endocervix	Only colour A recovered from the endocervix swabs
9	Correct UV gel recovered from the perianal	Only colour C recovered from the vulval and perianal swabs
10	Correct UV gel recovered from the rectum	Only colour B recovered from the rectum swabs
11	No contamination from the dummy found in the room or on the external areas of the dummy	No fluorescents within the room following completion of the assessment

Appendix B

Non-Intimate Touch DNA Recovery

Appendix B.1: Table 8
T-Test to compare the difference between the allele count recovery from the samples recovered from the underside of the wrist and the top side of the wrist.

Touch DNA T-Test - Allele Count				
T-Test of underside against topside wrist swabs (Allele Count)				
Statistics	**Underside of Wrist**	**Topside of Wrist**		
Average	16.6	18.71		
Standard Deviation	10.84	11.57		
Variance	117.4	133.81		
Count	8	8		
Coefficient of Variation	65.27185576	61.81		
Confidence	0.95	0.05		
F-Test				
F-Calculated	0	F table	19	0
***t*-Test Equal Variances**				
Spool	5.99			
t Calculated	2.79			
t table	2.14	**The difference is significant**		
***t*-Test unequal variances**				
DF	13.94			
t calculated	0.38			
t table	2.160368656	**The difference is not significant**		

Appendix B.2: Table 9
T-Test to compare the difference between the allele count recovery from the samples recovered in phase 1 and phase 2.

Touch DNA T-Test - Allele Count				
T-Test of underside against topside wrist swabs (Allele Count)				
Statistics	**Phase 1**	**Phase 2**		
Average	16.88	24.60		
Standard Deviation	10.53	14.03		
Variance	110.78	196.80		
Count	8	8		
Coefficient of Variation	62.37	57.03		
Confidence	0.95	0.05		
F-Test				
F-Calculated	0	F table	19	0
***t*-Test Equal Variances**				
Spool	6.63			
t Calculated	9.81			
t table	2.14	**The difference is significant**		
***t*-Test unequal variances**				
DF	12.98			
t calculated	1.25			
t table	2.17881283	**The difference is not significant**		

Appendix C

Non-Intimate Touch DNA Recovery ANOVA Calculations

Appendix C: Table 10
Non-Intimate Touch DNA Recovery ANOVA Calculations of all post 'perpetrator' contact swabs recovered from the 'victim'.

ANOVA Summary					
Source	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean Square (MS)	F-Statistical Value	P-Value
Between Groups	4	710.1136	177.5284	1.4677	0.2556
Within Groups	17	2056.2465	120.9557		
Total	21	2766.36.1			

Appendix D

Intimate DNA Recovery Results

Appendix D: Table 11
All Intimate DNA recovery results from all 4 SARCs

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/µl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
A/1	Control Substrate	n/a	Vulval	Pre-seeding	FCN	EZ1 Touch Swab	0.0003		1		Contaminant insufficient for comparison	n/a
A/1	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
A/1	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0004	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
A/1	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 5	Seminal: 0	Contaminant insufficient for comparison	n/a
A/1	Control Substrate	n/a	Perianal	Pre-seeding	FCN	EZ1 Touch Swab	0		1		Contaminant insufficient for comparison	n/a
A/1	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0004	Seminal: 0	Epithelial: 16	Seminal: 6	Contaminant insufficient for comparison	n/a
A/1	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0012	Seminal: 0	Epithelial: 23	Seminal: 1	Unsourced contaminant	n/a
A/1	Saliva	B: 1 in 2	Vulval	Post-seeding	FHP 1	EZ1 - Saliva Swab	0.0125		PCR1 = 41 PCR2 = 42 PCR3 = 41		Expected saliva donor profile plus unsourced contaminant (FHP 1 control unavailable for comparison)	Not met
A/1	n/a	n/a	Low vaginal	Post-seeding	FHP 1	Fast Diff Both Fractions	Epithelial: 0.0017	Seminal: 0	Epithelial: 30	Seminal: 0	Unsourced contaminant (FHP 1 control unavailable for comparison)	Not met
A/1	Semen	A: 1 in 50	High vaginal	Post-seeding	FHP 1	Fast Diff Both Fractions	Epithelial: 0.0013	Seminal: 0.0683	Epithelial: 11	Seminal: 32	Expected semen donor profile and least one further contributor not suitable for comparison	Met
A/1	Semen	A: 1 in 50	Endocervix	Post-seeding	FHP 1	Fast Diff Both Fractions	Epithelial: 0.0078	Seminal: 0.0856	Epithelial: 34	Seminal: 32	Expected semen donor profile and least one further contributor not suitable for comparison	Met
A/1	Saliva	B: 1 in 2	Perianal	Post-seeding	FHP 1	EZ1 - Saliva Swab	0.0078		38		Expected saliva donor profile and least one further	Met

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Appendix D: Table 11 (*continued*)

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/μl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
											contributor not suitable for comparison	
A/1	n/a	n/a	Anal Canal	Post-seeding	FHP 1	Fast Diff Both Fractions	Epithelial: 0.0079	Seminal: 0.0308	Epithelial: 35	Seminal: 32	Semen donor profile plus at least one further contributor not suitable for comparison	Met
A/1	Semen	A: 1 in 50	Rectum	Post-seeding	FHP 1	Fast Diff Both Fractions	Epithelial: 0.0005	Seminal: 0.0013	Epithelial: PCR1 = 13 PCR2 = 10 PCR3 = 7	Seminal: 21	Expected semen donor profile plus saliva donor profile. Additional component, insufficient for comparison	Not met
A/2	Control Substrate	n/a	Vulval	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
A/2	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0003	Seminal: 0	Epithelial: 1	Seminal: 0	Contaminant insufficient for comparison	n/a
A/2	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0002	Seminal: 0	Epithelial: 7	Seminal: 0	Contaminant insufficient for comparison	n/a
A/2	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0005	Seminal: 0	Epithelial: 7	Seminal: 0	Contaminant insufficient for comparison	n/a
A/2	Control Substrate	n/a	Perianal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0003	Seminal:0	Epithelial: PCR1 = 16 PCR2 = 5 PCR3 = 5	Seminal: 0	Unsourced contaminant	n/a
A/2	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0011	Seminal: 0	Epithelial: 25	Seminal: 0	Unsourced contaminant	n/a
A/2	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0004	Seminal: 0.0001	Epithelial: 5	Seminal: 0	Contaminant insufficient for comparison	n/a
A/2	Semen	A: 1 in 50	Vulval	Post-seeding	FHP 2	Fast Diff Both Fractions	Epithelial: 0.0017	Seminal: 0.0026	Epithelial: 27	Seminal: 30	Expected semen donor profile and least one further contributor not suitable for comparison	Met
A/2	n/a	n/a	Low vaginal	Post-seeding	FHP 2	Fast Diff Both Fractions	Epithelial: 0.0014	Seminal: 0.0052	Epithelial: 30	Seminal: 32	Semen donor profile and least one further contributor not suitable for comparison	Met
A/2	Saliva	B: 1 in 2	High vaginal	Post-seeding	FHP 2	Fast Diff Both Fractions	Epithelial: 0.0006	Seminal: 0.0013	Epithelial: PCR1 = 9 PCR2 = 9 PCR3 = 7	Seminal: 29	Expected saliva donor profile plus semen donor contaminant and least one further contributor not suitable for comparison	Not met
A/2	Saliva	B: 1 in 2	Endocervix	Post-seeding	FHP 2	Fast Diff Both Fractions	Epithelial: 0.0306	Seminal: 0.0003	Epithelial: 32	Seminal: 4	Expected saliva donor profile	Met
A/2	Semen	A: 1 in 50	Perianal	Post-seeding	FHP 2	Fast Diff Both Fractions	Epithelial: 0.0003	Seminal: 0.0118	Epithelial: 2	Seminal: 32	Expected semen donor profile	Met
A/2	n/a	n/a	Anal Canal	Post-seeding	FHP 2	Fast Diff Both Fractions	Epithelial: 0.0092	Seminal: 0.0006	Epithelial: 33	Seminal: PCR1 = 18 PCR2 = 18 PCR3 = 7	Saliva donor profile plus contaminant from volunteer B (responsible for model cleaning, and set-up)	Met
A/2	Saliva	B: 1 in 2	Rectum	Post-seeding	FHP 2	Fast Diff Both Fractions	Epithelial: 0.0242	Seminal: 0	Epithelial: 33	Seminal: 2	Expected saliva donor profile plus at least one further contributor not suitable for comparison	Met

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Appendix D: Table 11 (*continued*)

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/µl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
A/3	Control Substrate	n/a	Vulval	Pre-seeding	FCN	EZ1 Touch Swab	0.0001		1		Contaminant insufficient for comparison	n/a
A/3	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0005	Seminal: 0	Epithelial: 9	Seminal: 0	Unsourced contaminant	n/a
A/3	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0004	Seminal: 0	Epithelial: 3	Seminal: 0	Contaminant insufficient for comparison	n/a
A/3	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0009	Seminal: 0	Epithelial: 17	Seminal: 0	Unsourced contaminant	n/a
A/3	Control Substrate	n/a	Perianal	Pre-seeding	FCN	EZ1 Touch Swab	0.0006		16		Unsourced contaminant	n/a
A/3	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0002	Seminal: 0	Epithelial: 4	Seminal: 0	Contaminant insufficient for comparison	n/a
A/3	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0003	Seminal: 0	Epithelial: 1	Seminal: 0	Contaminant insufficient for comparison	n/a
A/3	Saliva	B: 1 in 2	Vulval	Post-seeding	FHP 3	EZ1-Saliva Swab	0.0217		34		Expected saliva donor profile plus at least one further contributor not suitable for comparison	Met
A/3	n/a	n/a	Low vaginal	Post-seeding	FHP 3	Fast Diff Both Fractions	Epithelial: 0.0002	Seminal: 0	Epithelial: 4	Seminal: 0	Trace contamination insufficient for comparison	Met
A/3	Semen	A: 1 in 50	High vaginal	Post-seeding	FHP 3	Fast Diff Both Fractions	Epithelial: 0.0023	Seminal: 0.0182	Epithelial: 32	Seminal: 32	Expected semen donor profile plus saliva donor contaminant and at least one further contributor not suitable for comparison	Not met
A/3	Semen	A: 1 in 50	Endocervix	Post-seeding	FHP 3	Fast Diff Both Fractions	Epithelial: 0.0045	Seminal: 0.0049	Epithelial: 34	Seminal: 37	Expected semen donor profile plus at least one further contributor not suitable for comparison	Met
A/3	Saliva	B: 1 in 2	Perianal	Post-seeding	FHP 3	EZ1 - Saliva Swab	0.0133		34		Expected saliva donor profile plus at least one further contributor not suitable for comparison	Met
A/3	n/a	n/a	Anal Canal	Post-seeding	FHP 3	Fast Diff Both Fractions	Epithelial: 0.0004	Seminal: 0	Epithelial: PCR1 = 11 PCR2 = 12 PCR3 = 8	Seminal: 0	Cannot exclude saliva donor profile, plus at least one further contributor not suitable for comparison	Met
A/3	Semen	A: 1 in 50	Rectum	Post-seeding	FHP 3	Fast Diff Both Fractions	Epithelial: 0.0002	Seminal: 0	Epithelial: 7	Seminal: 0	Unsourced contaminant, and seeded DNA not detected	Not met
B/1	Control Substrate	n/a	Vulval	Pre-seeding	FCN	EZ1 - Touch Swab	0		0		No detectable background contamination	n/a
B/1	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
B/1	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
B/1	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
B/1	Control Substrate	n/a	Perianal	Pre-seeding	FCN	EZ1 - Touch Swab	0		0		No detectable background contamination	n/a

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Appendix D: Table 11 (*continued*)

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/µl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
B/1	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 6	Seminal: 0	Contaminant insufficient for comparison	n/a
B/1	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
B/1	Saliva	B: 1 in 2	Vulval	Post-seeding	FHP 4	EZ1 - Saliva Swab	0.0189		33		Expected semen donor profile plus at least one further contributor not suitable for comparison	Met
B/1	n/a	n/a	Low vaginal	Post-seeding	FHP 4	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	Met
B/1	Semen	A: 1 in 2	High vaginal	Post-seeding	FHP 4	Fast Diff - Both Fractions	Epithelial: 0.0103	Seminal: 0.372	Epithelial: 33	Seminal: 33	Expected semen donor profile plus at least one further contributor not suitable for comparison	Met
B/1	Semen	A: 1 in 2	Endocervix	Post-seeding	FHP 4	Fast Diff - Both Fractions	Epithelial: 0.2741	Seminal: 1.0141	Epithelial:32	Seminal: 33	Expected semen donor profile plus at least one further contributor not suitable for comparison	Met
B/1	Saliva	B: 1 in 2	Perianal	Post-seeding	FHP 4	EZ1 - Saliva Swab	0.0765		32		Expected saliva donor profile	Met
B/1	n/a	n/a	Anal Canal	Post-seeding	FHP 4	Fast Diff - Both Fractions	Epithelial: 0.003	Seminal: 0.016	Epithelial:32	Seminal: 34	Expected semen donor profile plus at least one further contributor not suitable for comparison	Met
B/1	Semen	A: 1 in 2	Rectum	Post-seeding	FHP 4	Fast Diff - Both Fractions	Epithelial: 0.2732	Seminal: 0.3669	Epithelial: 33	Seminal: 32	No detectable background contamination	Met
B/2	Control Substrate	n/a	Vulval	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
B/2	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
B/2	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
B/2	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 0	Seminal:0	No detectable background contamination	n/a
B/2	Control Substrate	n/a	Perianal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 1	Seminal: 0	Contaminant insufficient for comparison	n/a
B/2	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 0	Seminal:0	No detectable background contamination	n/a
B/2	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 0	Seminal:0	No detectable background contamination	n/a
B/2	Semen	A: 1 in 2	Vulval	Post-seeding	FHP 5	Fast Diff - Both Fractions	Epithelial: 0.3095	Seminal: 1.0408	Epithelial: 32	Seminal: 32	Expected semen donor profile	Met
B/2	n/a	n/a	Low vaginal	Post-seeding	FHP 5	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 1	Seminal: 0	Contaminant insufficient for comparison	Met
B/2	Saliva	B: 1 in 2	High vaginal	Post-seeding	FHP 5	Fast Diff - Both Fractions	Epithelial: 0.0339	Seminal: 0	Epithelial: 32	Seminal: 1	Expected saliva donor profile plus at least one further contributor not suitable for comparison	Met
B/2	Saliva	B: 1 in 2	Endocervix	Post-seeding	FHP 5	Fast Diff - Both Fractions	Epithelial: 0.0628	Seminal: 0	Epithelial: 32	Seminal: 4	Expected saliva donor profile plus at least one further	Met

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Appendix D: Table 11 (*continued*)

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/µl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
											contributor not suitable for comparison	
B/2	Semen	A: 1 in 2	Perianal	Post-seeding	FHP 5	Fast Diff - Both Fractions	Epithelial: 0.2622	Seminal: 1.0371	Epithelial: 32	Seminal: 32	Expected semen donor profile	Met
B/2	n/a	n/a	Anal Canal	Post-seeding	FHP 5	Fast Diff - Both Fractions	Epithelial: 0.0007	Seminal: 0.0007	Epithelial: 19	Seminal: 21	Saliva donor profile plus at least one further contributor not suitable for comparison	Met
B/2	Saliva	B: 1 in 2	Rectum	Post-seeding	FHP 5	Fast Diff - Both Fractions	Epithelial: 0.0015	Seminal: 0	Epithelial: 21	Seminal: 4	Expected saliva donor profile plus at least one further contributor not suitable for comparison	Met
C/1	Control Substrate	n/a	Vulval	Pre-seeding	FCN	EZ1 - Touch Swab	0		0		No detectable background contamination	n/a
C/1	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0	Seminal: 0.0001	Epithelial: 0	Seminal: 2	Contaminant insufficient for comparison	n/a
C/1	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0.0002	Seminal: 0.0001	Epithelial: 0	Seminal: 2	Contaminant insufficient for comparison	n/a
C/1	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0	Seminal: 0.0001	Epithelial: 0	Seminal: 7	Unsourced contaminant	n/a
C/1	Control Substrate	n/a	Perianal	Pre-seeding	FCN	EZ1 - Touch Swab	0		0		No detectable background contamination	n/a
C/1	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 1	Seminal: 0	Contaminant insufficient for comparison	n/a
C/1	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 2	Contaminant insufficient for comparison	n/a
C/1	Saliva	B: undiluted	Vulval	Post-seeding	FHP 6	EZ1 - Touch Swab	0.0722		32		Expected saliva donor profile	Met
C/1	n/a	n/a	Low vaginal	Post-seeding	FHP 6	Fast Diff Both Fractions	Epithelial: 0.0064	Seminal: 0.0509	Epithelial: 47	Seminal: 32	Semen donor profile and saliva donor profile	Met
C/1	Semen	A: undiluted	High vaginal	Post-seeding	FHP 6	Fast Diff Both Fractions	Epithelial: 0.0009	Seminal: 2.4133	Epithelial: 30	Seminal: 33	Expected semen donor profile and at least one further contributor not suitable for comparison	Met
C/1	Semen	A: undiluted	Endocervix	Post-seeding	FHP 6	Fast Diff Both Fractions	Epithelial: 0.2425	Seminal: 1.7339	Epithelial: 32	Seminal: 33	Expected semen donor profile and at least one further contributor not suitable for comparison	Met
C/1	Saliva	B: undiluted	Perianal	Post-seeding	FHP 6	EZ1 - Touch Swab	0.027		33		Expected saliva donor profile and least one further contributor not suitable for comparison	Met
C/1	n/a	n/a	Anal Canal	Post-seeding	FHP 6	Fast Diff Both Fractions	Epithelial: 0.0077	Seminal: 1.2379	Epithelial: 34	Seminal: 32	Semen donor profile and at least one further contributor not suitable for comparison	Met
C/1	Semen	A: undiluted	Rectum	Post-seeding	FHP 6	Fast Diff Both Fractions	Epithelial: 0.443	Seminal: 3.424	Epithelial: 32	Seminal: 32	Expected semen donor profile	Met
C/2	Control Substrate	n/a	Vulval	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a

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Appendix D: Table 11 (*continued*)

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/µl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
C/2	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 1	Contaminant insufficient for comparison	n/a
C/2	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 3	Contaminant insufficient for comparison	n/a
C/2	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
C/2	Control Substrate	n/a	Perianal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
C/2	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0.0001	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
C/2	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff - Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 2	Seminal: 0	Contaminant insufficient for comparison	n/a
C/2	Semen	A: undiluted	Vulval	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.7407	Seminal: 1.9003	Epithelial: 33	Seminal: 32	Expected semen donor profile and least one further contributor not suitable for comparison	Met
C/2	n/a	n/a	Low vaginal	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0	Seminal: 0.0003	Epithelial: 0	Seminal: 2	Contributor insufficient for comparison	Met
C/2	Saliva	B: undiluted	High vaginal	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.001	Seminal: 0.0009	Epithelial: 7	Seminal: 30	Expected saliva donor profile plus unexpected semen donor profile	Not met
C/2 (repeat exercise)	Saliva	B: undiluted	High vaginal	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.0002	Seminal: 0	Epithelial: 4	Seminal: 7	Expected saliva donor profile plus unsourced partial profile	Not met
C/2	Saliva	B: undiluted	Endocervix	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.0226	Seminal: 0	Epithelial: 32	Seminal: 0	Expected saliva donor profile	Met
C/2	Semen	A: undiluted	Perianal	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.0208	Seminal: 0.0572	Epithelial: 33	Seminal: 32	Expected semen donor profile and at least one further contributor not suitable for comparison	Met
C/2	n/a	n/a	Anal Canal	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.012	Seminal: 0	Epithelial: 34	Seminal: 1	Saliva donor profile and least one further contributor not suitable for comparison	Met
C/2	Saliva	B: undiluted	Rectum	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.0066	Seminal: 0.0002	Epithelial: 36	Seminal: 9	Expected saliva donor profile plus unexpected semen donor profile and least one further contributor not suitable for comparison	Not met
C/2 (repeat exercise)	Saliva	B: undiluted	Rectum	Post-seeding	FHP 7	Fast Diff - Both Fractions	Epithelial: 0.0326	Seminal: 0	Epithelial: 32	Seminal: 1	Expected saliva donor profile and least one further contributor not suitable for comparison	Met
C/3	Control Substrate	n/a	Vulval	Pre-seeding	FCN	EZ1 - Touch Swab	0		0		No detectable background contamination	n/a
C/3	Control Substrate	n/a	Low vaginal	Pre-seeding	FCN	Fast Diff- Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
C/3	Control Substrate	n/a	High vaginal	Pre-seeding	FCN	Fast Diff- Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a

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Appendix D: Table 11 (*continued*)

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/µl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
C/3	Control Substrate	n/a	Endocervix	Pre-seeding	FCN	Fast Diff-Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
C/3	Control Substrate	n/a	Perianal	Pre-seeding	FCN	EZ1 - Touch Swab	0		0		No detectable background contamination	n/a
C/3	Control Substrate	n/a	Anal Canal	Pre-seeding	FCN	Fast Diff-Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 7	Seminal: 0	Profile match to volunteer 2 responsible for cleaning and model set-up	n/a
C/3	Control Substrate	n/a	Rectum	Pre-seeding	FCN	Fast Diff-Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	n/a
C/3	Saliva	B: undiluted	Vulval	Post-seeding	FHP 8	EZ1 - Touch Swab	0.0689		32		Expected saliva donor profile	Met
C/3	n/a	n/a	Low vaginal	Post-seeding	FHP 8	Fast Diff-Both Fractions	Epithelial: 0.0455	Seminal: 0.6651	Epithelial: 32	Seminal: 33	Semen donor profile and least one further contributor not suitable for comparison	Met
C/3	Semen	A: undiluted	High vaginal	Post-seeding	FHP 8	Fast Diff-Both Fractions	Epithelial: 0.0173	Seminal: 0.8664	Epithelial: 34	Seminal: 32	Expected semen donor profile and least one further contributor not suitable for comparison	Met
C/3	Semen	A: undiluted	Endocervix	Post-seeding	FHP 8	Fast Diff-Both Fractions	Epithelial: 0.7991	Seminal: 5.2806	Epithelial: 33	Seminal: 32	Expected semen donor profile and least one further contributor not suitable for comparison	Met
C/3	Saliva	B: undiluted	Perianal	Post-seeding	FHP 8	EZ1 - Touch Swab	0.0523		34		Expected saliva donor profile and least one further contributor not suitable for comparison	Met
C/3	n/a	n/a	Anal Canal	Post-seeding	FHP 8	Fast Diff-Both Fractions	Epithelial: 0.3772	Seminal: 0.8274	Epithelial: 35	Seminal: 33	Semen donor profile and least one further contributor not suitable for comparison	Met
C/3	Semen	A: undiluted	Rectum	Post-seeding	FHP 8	Fast Diff-Both Fractions	Epithelial: 0.0211	Seminal: 1.3175	Epithelial: 33	Seminal:33	Expected semen donor profile and least one further contributor not suitable for comparison	Met
D/1	Saliva	B: undiluted	Vulval	Post-seeding	FHP 9	EZ1 - Touch Swab	0.0181		32		Expected semen donor profile	Met
D/1	n/a	n/a	Low vaginal	Post-seeding	FHP 9	Fast Diff-Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	Met
D/1	Semen	A: undiluted	High vaginal	Post-seeding	FHP 9	Fast Diff-Both Fractions	Epithelial: 0.0003	Seminal: 0.0002	Epithelial: 3	Seminal: 5	Insufficient for comparison	Not met
D/1	Semen	A: undiluted	Endocervix	Post-seeding	FHP 9	Fast Diff-Both Fractions	Epithelial: 0.0006	Seminal: 0.005	Epithelial: 14	Seminal: 14	Expected semen donor profile and least one further contributor not suitable for comparison	Met
D/1	Saliva	B: undiluted	Perianal	Post-seeding	FHP 9	EZ1 - Touch Swab	0.0344		32		Expected saliva donor profile	Met
D/1	n/a	n/a	Anal Canal	Post-seeding	FHP 9	Fast Diff-Both Fractions	Epithelial: 0	Seminal 0.0003	Epithelial: 0	Seminal: 0	No detectable background contamination	Met
D/1	Semen	A: undiluted	Rectum	Post-seeding	FHP 9	Fast Diff-Both Fractions	Epithelial:0.0005	Seminal 0.0005	Epithelial: 13	Seminal: 5	Expected semen donor profile and least one further contributor not	Met

(*continued on next page*)

Appendix D: Table 11 (*continued*)

SARC/Model	Expt./ Cellular Material	Donor/ Dilution in PBS	Location/ Area Model 1	Time	Taken by:	Extraction Type	Quant Score (ng/µl)		Allele Count		Result: Comparison to Sample/ Conclusion	Acceptance criteria met/ not met
											suitable for comparison	
D/2	Semen	A: undiluted	Vulval	Post-seeding	FHP 10	Fast Diff Both Fractions	Epithelial: 0.0021	Seminal: 0.0062	Epithelial: 17	Seminal: 31	Expected semen donor profile and least one further trace level contributor insufficient for comparison	Met
D/2	n/a	n/a	Low vaginal	Post-seeding	FHP 10	Fast Diff Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 3	Seminal: 1	Insufficient for comparisons	Met
D/2	Saliva	B: undiluted	High vaginal	Post-seeding	FHP 10	Fast Diff Both Fractions	Epithelial: 0.0331	Seminal: 0	Epithelial: 32	Seminal: 0	Expected saliva donor profile and least one further trace level contributor insufficient for comparison	Met
D/2	Saliva	B: undiluted	Endocervix	Post-seeding	FHP 10	Fast Diff Both Fractions	Epithelial: 0.0429	Seminal: 0	Epithelial: 32	Seminal: 0	Expected saliva donor profile and least one further trace level contributor insufficient for comparison	Met
D/2	Semen	A: undiluted	Perianal	Post-seeding	FHP 10	Fast Diff Both Fractions	Epithelial: 0.0027	Seminal: 0.119	Epithelial: 27	Seminal: 33	Expected semen donor profile and least one further trace level contributor insufficient for comparison	Met
D/2	n/a	n/a	Anal Canal	Post-seeding	FHP 10	Fast Diff Both Fractions	Epithelial: 0.0001	Seminal: 0.0004	Epithelial: 3	Seminal: 5	Contaminant insufficient for comparisons	Met
D/2	Saliva	B: undiluted	Rectum	Post-seeding	FHP 10	Fast Diff Both Fractions	Epithelial: 0.0065	Seminal: 0	Epithelial: 32	Seminal: 0	Expected saliva donor profile and least one further trace level contributor insufficient for comparison	Met
D/3	Saliva	B: undiluted	Vulval	Post-seeding	FHP 11	EZ1 - Touch Swab	0.0632		32		Expected saliva donor profile	Met
D/3	n/a	n/a	Low vaginal	Post-seeding	FHP 11	Fast Diff Both Fractions	Epithelial: 0.0001	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable background contamination	Met
D/3	Semen	A: undiluted	High vaginal	Post-seeding	FHP 11	Fast Diff Both Fractions	Epithelial: 0	Seminal: 0	Epithelial: 0	Seminal: 0	No detectable profile	Not met
D/3	Semen	A: undiluted	Endocervix	Post-seeding	FHP 11	Fast Diff Both Fractions	Epithelial: 0.0033	Seminal: 0.0035	Epithelial: 30	Seminal: 31	Expected semen donor profile and least one further trace level contributor insufficient for comparison	Met
D/3	Saliva	B: undiluted	Perianal	Post-seeding	FHP 11	EZ1 - Touch Swab	0.0242		34		Expected saliva donor profile and least one further trace level contributor insufficient for comparison	Met
D/3	n/a	n/a	Anal Canal	Post-seeding	FHP 11	Fast Diff-Both Fractions	Epithelial: 0.0001	Seminal: 0.0001	Epithelial: 4	Seminal: 6	Insufficient for comparisons	Met
D/3	Semen	A: undiluted	Rectum	Post-seeding	FHP 11	Fast Diff-Both Fractions	Epithelial: 0.002	Seminal: 0.0002	Epithelial: 36	Seminal: 10	Expected semen donor profile plus saliva donor profile and least one further trace level contributor insufficient for comparison	Not met

References

[1] T. Walker, R. Majeed-Ariss, R. Riley, C. White, Women's experiences of attending an English sexual assault referral centre: an exploratory study, J. Forensic Psychiatr. Psychol. (2020) 123–136. https://www.tandfonline.com/doi/full/10.1080/14789949.2019.1683217.

[2] B. Ludes, A. Geraut, M. Vali, D. Cusack, D. Ferrara, E. Keller, P. Mangin, D. Vieira, Guidelines examination of victims of sexual assault harmonization of forensic and medico-legal examination of persons, Int. J. Leg. Med. 132 (6) (2018) 1671–1674, https://doi.org/10.1007/s00414-018-1791-y.

[3] NHS National Service Specification for Sexual Assault Referral Centres Incorporating NHS Public Health Functions Agreement 2023-24 Version 1, 26 June 2023. PRN00577-national-service-specification-sexual-assault-referral-centres-updated.pdf. (Accessed 31 December 2024).

[4] Rebecca Bihler Miscarriages in the British legal system, The Guildford Four and the Birmingham Six, Grin Publishings, 2016 [Book] 10/06/.

[5] Evidence Based Justice Lab: Psychology, Data Science and Law at the University of Exter Law School. Miscarriages of Justice Registry - Evidenced Based Justice Lab - University of Exeter [Accessed 31 December 2024].

[6] L. Elks, Ten years of the criminal cases review commission RIGHTING MISCARRIAGES OF JUSTICE?. https://files.justice.org.uk/wp-content/uploads/2016/04/06170741/Righting-Miscarriages-of-Justice.pdf, 2008. (Accessed 25 February 2025).

[7] L. Kelly, J. Lovett, L. Regan. Home Office Research Study 293 A Gap or a Chasm? Attrition in Reported Rape Cases. Jan 2005 (PDF) Home Office Research Study 293 A Gap or a Chasm? Attrition in Reported Rape Cases [Accessed 31 December 2024].

[8] Parliament.uk, House of commons hansard ministerial statements for 12 July 2007 (pt 0002), Column 67WS, https://publications.parliament.uk/pa/cm200607/cmhansrd/cm070712/wmstext/70712m0002.htm, 2025. (Accessed 25 February 2025).

[9] Legislation.gov.uk, Forensic science regulator act 2021. https://www.legislation.gov.uk/ukpga/2021/14/contents, 2021. (Accessed 25 February 2025).

[10] Parliament.uk, Royal assent - hansard - UK parliament. https://hansard.parliament.uk/lords/2021-04-29/debates/6B29F4C1-4077-4A0D-8A4C-9E0D8324A14B/RoyalAssent, 2025. (Accessed 25 February 2025).

[11] Forensic Science Regulator Code of Practice, Version 1 Forensic Science Regulator Code of Practice- March 2023 V1.pdf (cjx.gov.uk), March 2023. (Accessed 27 December 2024).

[12] HM Government: The end-to-end Rape Review on Findings and Actions June 2021 the end-to-end Rape Review Report on Findings and Actions[Accessed 31 December 2024].

[13] FSR-L-B10, Contamination in a sexual assault referral centre. Lessons learnt, Issue 10, www.gov.uk/government/publications/forensic-science-lessons-learnt-issue-10, 2020. (Accessed 27 December 2024).

[14] FSR-GUI-0017 Guidance, DNA Contamination Controls – Forensic Medical Examinations, January 2024. Issue 1. (Accessed 27 December 2024).

[15] FSR-GUI-0020 Guidance, Forensic Medical Examination of Sexual Offence Complainants, January 2024. Issue 1. (Accessed 27 December 2024).

[16] ISO, ISO 15189:2022 medical laboratories – requirements for quality and competence [online] ISO, https://www.iso.org/standard/76677.html, 2022. (Accessed 27 December 2024).

[17] FFLM, Recommendations for the Collection of Forensic Specimens from Complainants and Suspects - FFLM, 2024 [online] Available at: Recommendations for the Collection of Forensic Specimens from Complainants and Suspects - FFLM/. (Accessed 27 December 2024).

[18] FFLM, Recommendations for the collection of forensic specimens from complainants and suspects – the evidence - FFLM. https://fflm.ac.uk/resources/publications/recommendations-for-the-collection-of-forensic-specimens-from-complainants-and-suspects-the-evidence/, 2024. (Accessed 26 December 2024).

[19] Forensic science regulator guidance validation FSR-G-201 issue 2. (n.d.). Available at: https://assets.publishing.service.gov.uk/media/5f6b1a3de90e077ca292204f/201_-_FSR-G-201_Validation_Guidance_Issue_2.pdf. [Accessed 31 December 2024].

[20] Revised ILAC-G19 document published international laboratory accreditation cooperation. https://ilac.org/latest_ilac_news/revised-ilac-g19-document-published/. (Accessed 31 December 2024).

[21] V. Kallupurackal, S. Kummer, P. Voegeli, A. Kratzer, G. Dorum, C. Haas, S. Hess, Sampling touch DNA from human skin following skin-to -skin contact in mock assault scenarios—A comparison of nine collection methods, J. Forensic Sci. 66 (2021) 1889–1900.

[22] E. Meixner, V. Kallupurackal, A. Kratzer, P. Voegeli, M. Thali, S. Bolliger, Persistence and detection of touch DNA and blood stain DNA on pig skin exposed to water, Forensic Sci. Med. Pathol. 16 (2) (2020) 243–251, https://doi.org/10.1007/s12024-020-00234-3.

[23] T.J Verdon, R.J. Mitchell, R.A.H. van Oorschot, Swabs as DNA collection devices for sampling different biological materials from different substrates, J. Forensic Sci. 59 (2014) 1080–1089, https://doi.org/10.1111/1556-4029.12427.

[24] A.J. Loeve, A.C. Bilo, E. Emirdag, M. Sharify, F.M. Jansen, J. Dankelman, *In Vitro* validation of vaginal sampling in rape victims: the problem of Locard's principle, Forensic Sci. Med. Pathol. 9 (2) (2013) 154–162, https://doi.org/10.1007/s12024-013-9426-6.

[25] D. Sweet, M. Lorente, A. Valenzuela, E. Vilanueva, An improved method to recover saliva from human skin: the double swab technique, J. Forensic Sci. 42 (2) (1997) 14120J, https://doi.org/10.1520/jfs14120j.

[26] B.C.M. Pang, B. K, K. Cheung, Double swab technique for collecting touched evidence, Leg. Med. 9 (4) (2007) 181–184, https://doi.org/10.1016/j.legalmed.2006.12.003.

[27] A.E. Fonnelop, M. Ramse, T. Egeland, P. Gill, The implications of shedder status and background DNA on direct and secondary transfer in an attack scenario, Forensic. Sci. Int. Genet. 29 (2017) 48–60, https://doi.org/10.1016/j.fsigen.2017.03.019.

[28] A. Lowe, C. Murray, J. Whitaker, G. Tully, P. Gill, The propensity of individuals to deposit DNA and secondary transfer of low-level DNA from individuals to inert surfaces, Forensic Sci. Int. 129 (1) (2002) 25–34, https://doi.org/10.1016/s0379-0738(02)00207-4.

[29] FFLM, Recommendations on the order of ano-genital sampling when obtaining forensic specimens from complainants and suspects of sexual offences - FFLM. https://fflm.ac.uk/resources/publications/recommendations-on-the-order-of-ano-genital-sampling-when-obtaining-forensic-specimens-from-complainants-and-suspects-of-sexual-offences/, 2024. (Accessed 28 December 2024).