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Research Paper

Understanding and mitigating the risks that environmental DNA contamination poses to the recovery of forensic evidence from victims and suspects of rape and sexual assault

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ABSTRACT

The high sensitivity of current DNA analysis technologies poses significant anti-contamination challenges when recovering evidence from individuals in sexual assault referral centres (SARC)s and police custody forensic medical examination rooms where background DNA levels cannot be controlled as effectively as within a DNA laboratory setting. In response to reported instances of DNA evidence becoming compromised during recovery within SARCs, the UK Forensic Science Regulator (FSR) established anti-contamination guidelines for these facilities including requirements for environmental monitoring (EM) and target levels for air replacement with a view to managing the risk of air-borne contamination.

Forensic samples were recovered from different volunteers during 24 forensic medical examinations across four SARC and four police custody suites, all utilising different cleaning and air replacement regimes. Of the 144 EM samples taken from high contamination risk areas of the forensic medical room, DNA was present in 84 % of these swabs. Significantly less DNA was found to be present in the SARC when compared to custody suites.

Despite the high environmental DNA levels observed, none resulted in contamination of the forensic evidence recovered from the volunteer patients. This study assessed the real-life risk to evidential samples and the results demonstrated that provided appropriate anti-contamination measures were used in recovering and handling evidential samples, the risk of DNA contamination was effectively managed, even within facilities displaying high background levels of DNA. The results from this assessment of risk have enabled target levels of cleanliness to be defined in FSR guidelines[1] together with revised requirements for airflow rates.

1. Introduction

Complainants of rape and sexual assault who are referred to a SARC may undergo a forensic medical examination, conducted within a dedicated forensic medical examination room where items or samples of potential forensic evidential value are recovered as part of the criminal investigation of the alleged offence. DNA results are key to evidencing contact between the victim and an assailant: in 15–20 % of crime cases, forensic science is the only method used to identify a person of interest, to generate a line of enquiry or to secure a guilty plea,[2] therefore DNA evidence is critical in supporting the victim's criminal case. Home Office data on crime outcomes in England and Wales for 2021 showed that 1.3 % (991 charges) of the recorded rape offences that were assigned an outcome resulted in a charge or summons,[2] which has since risen in the year ending 2022 to 2.1 % (1401 charges).[3] These statistics highlight the importance of forensic evidence in sexual assault and rape cases and the significance of the role of the SARC services, they also reflect the increased focus on rape following the cross-criminal justice system rape review.[4]

The sensitivity of forensic DNA testing has significantly improved since the introduction of multiplex Short Tandem Repeat (STR) analysis, but the enhanced sensitivity of current DNA testing technologies brings with it an increased risk of detecting contamination, which could potentially lead to a miscarriage of justice.[5] Consequently, it is essential that robust anti-contamination measures are in place within the SARCs to minimise the risk of compromising the forensic evidence.

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Instances have been reported of DNA contamination in UK SARCs including the identification of DNA from a vaginal swab which matched a suspect from an unrelated case, the victim of which had been examined previously within the same SARC.[6] Although the root cause of the contamination was never identified, many failings in anti-contamination measures were found throughout the investigation. In addition to the risks highlighted in this report, other concerns were raised directly with the FSR by the Crown Prosecution Service in 2013 and then by the Violence Against Women and Girls (VAWG) Programme, regarding flawed forensic evidence in several failed court cases. In response to these issues the FSR has put in place a quality framework to minimise the risk of such failures in the future. This framework comprises of two strands:

1) Accreditation of SARCs to the most appropriate international quality standard i.e. ISO15189: Medical Laboratories – Requirements for Quality & Competence,[7] and
2) The development of SARC-specific quality standards and guidelines within the FSR Code of Practice[8] to which the SARCs are required to comply[1,9] (previously[10,11&12]).

The latter detailed a number of measures to minimise the risks of DNA contamination including the validation of the cleaning methods to be used, monthly deep cleans, the use of personal protective equipment (PPE) and forensic DNA grade consumables, anticontamination training, access control, minimum air replacement rates and establishing a staff DNA elimination database. It is not yet fully understood what risks DNA contamination in the forensic medical examination room pose when all these measures are in place. A further anti-contamination requirement detailed in FSR-C-116 *Sexual Assault Examination: Requirements for the Assessment, Collection and Recording of Forensic Science Related Evidence*[10] was to conduct environmental monitoring sampling and to take advice and feedback from forensic service providers (FSP) to whom the samples are submitted for analysis. Unfortunately, this FSR document did not define what background levels were deemed acceptable within SARCs as no data was available at the time on the real-life risk posed by different background levels of DNA. This resulted in variation in the criteria set and advice given between FSPs. The most stringent approach has been to apply the same requirements to SARC EM results as those stipulated for DNA laboratory clean rooms, in which a red, amber or green status is applied to the observed EM results and for which red constitutes a "fail", when specified background levels are exceeded. This in turn triggered the temporary closure of facilities until cleaning, re-sampling and analysis demonstrates that an acceptable background level has been restored. This poses a problem because, whilst very strict control measures can be applied in DNA laboratories, these are unachievable in SARCs, where the welfare of the victim is of paramount importance. For example, only personnel in full PPE enter the DNA laboratory environment, but this is not possible in a SARC forensic medical examination room, where victims and their supporters are present without the requirement to wear PPE. Two studies have exemplified the huge challenges involved in attempting to meet these DNA lab-based background level requirements, including significant cost implications and a detrimental impact to complainants of rape and sexual assault.[5,13] It is anticipated that the FSR standards set for SARCs are likely to be replicated for the forensic medical examination of suspects of sexual assault in custody. Therefore, similar issues will have to be addressed in custodial environments which can face even greater challenges than SARCs in controlling their environments.

There is a risk to both SARC and custody services that they will be benchmarked against forensic DNA laboratory cleanliness standards. The purpose of this study is to investigate the risk of contamination from the SARC and custody forensic environments to the DNA evidence recovered within them. The aim is for the experimental data from this study to be used to support understanding of the risk of contamination to allow for informed decisions, based on data, on the most appropriate anticontamination regulatory requirements that are proportionate to the risks.

A further aim was to consider whether high air flow rates stipulated in the FSR Code of Practice[8] of greater than 20 whole room replacements per hour reduced the risk of samples becoming contaminated during recovery when compared with lower/no air replacements. The air exchange value in the FSR Code was based on best practice within certain clinical environments, but data was lacking on the effect airflow systems have on the recovery of DNA evidence and the contamination risk it poses. This requirement had been raised nationally as a risk to the SARC service providers due to the significant installation cost of such a system. To investigate the necessity of expensive, high-end, air replacement systems within the SARCs, air samples were taken, in addition to negative control (NC) samples and EM samples. Air filtration sampling systems have been used in a number of studies[14–17] to sample DNA from the environment, proving to be a sensitive, cost effective and non-invasive method for the characterisation of biodiversity and speciation. Air sampling has also been used in a recent study as a means of obtaining DNA evidence of human occupancy in indoor premises given that humans constantly shed DNA into the environment which may either remain suspended in the air or it settles onto surfaces as indoor dust.[17] This built on previous studies, including Toothman et al., which demonstrated that human DNA is present in indoor dust in sufficient quantity and quality to produce allele counts in STR analysis.[19] Also, the emerging field of using airborne environmental DNA for forensic applications has been recently reviewed.[20]

2. Method

In order to understand the current risk that DNA contamination poses within a forensic medical examination room an EM study was conducted which involved 2 phases:

Phase 1: Assessment of the levels of environmental DNA contamination within medical examination facilities and the contamination risk this poses to the evidential samples recovered
Phase 2: Assessment of the impact of air replacement rates on contamination risk and suitability of air sampling for environmental monitoring.

The facilities involved in each phase have been outlined in Table 1.

All swabs recovered within this study were done so following the wet and dry technique[21,22] which is widely used across all areas of forensics in the UK. Forensic DNA grade MW102 cotton swabs (SceneSafe) were used throughout this exercise. All swabs were used in duplicate with the first being moistened with forensic DNA grade water (SceneSafe) followed by a dry swab.

All forensic medical examination process conducted in this study were in line with FFLM recommendation guidance.[23,24]

2.1. Phase 1: assessment of background DNA levels and contamination risk

The aim of phase 1 of this study was to establish suitable criteria for determining acceptable levels of background DNA in a SARC and custody suite by assessing the realistic risk in established forensic medical environments and practices.

Three mock forensic medical examinations were carried out at each of the four SARC and four custodial facilities on different volunteers, totalling 24 examinations. Prior to attendance at these facilities, each volunteer washed their upper inner thigh, then dried the area with forensic DNA grade tissue and wrapped a sterile bandage around the area.

In advance of each mock examination, six moist and dry EM samples were taken from locations (listed in Appendix 1B) within the medical examination room that had been identified from previous research to

Table 1
A table listing the facilities involved in each phase of the study, the average usage, the cleaning product used, and whether EM is conducted to monitor the cleanliness of the forensic medical examination rooms.

Location type	Location reference	Phase Involvement	Air Flow: number of whole room replacement per hour	Average use per month	Routine EM	Cleaning Reagent
Custody	L01	1	0	*	No	Chemgene
Custody	L02	1	0	*	No	Chemgene
Custody	L03	1	0	*	No	Chemgene
Custody	L04	1	0	*	No	Chemgene
SARC	L05	1 & 2	~10	>30	No	Chemgene
SARC	L06	1 & 2	0	<10	No	Chemgene
SARC	L07	1	0	<10	No	Chemgene
SARC	L08	1	0	>30	Yes	Actichlor+
SARC	L09	2	>20	>30	No	Microsol
SARC	L10	2	>20	<10	No	Chemgene
Domestic House		2	0	N/A		
Public Toilet		2	0			
Public Bar		2	0			

*Custody medical examination rooms used in this study were multipurpose, including use for non forensic medical assessments.

commonly pose the greatest contamination risk.[14]

Following EM sampling, the Forensic Healthcare Practitioner (FHP), volunteer and Crisis Worker (in SARCs), or Custody Officer (in custody), then entered the examination room. The FHP first placed three negative control (NC) samples (1A, 1B & 1C) in the area the samples were to be prepared, i.e. on the sample trolley in the SARC and worksurface in custody. NC samples are swabs that have been moistened with forensic DNA grade water and placed, tip up, in a container, exposed to the air.

The volunteer changed out of their clothes (including the removal of the bandage, but not the removal of underwear) into a patient gown and sat on the examination couch. The FHP then conducted processes to mimic a medical examination, i.e. taking height and weight measurements, and explaining the process and what samples would be taken.

The FHP placed an additional three NC samples (2A, 2B & 2C) in the same location as the first set of NC samples (1A, 1B, & 1C). A moist and dry thigh swab was then recovered from the volunteer by the FHP, from the area of skin that had previously been cleaned and bandaged. Immediately after, the three NC samples (2A, 2B & 2C) were then placed back into their sample tubes. Next the FHP recovered a moist swab and dry swab of each of the volunteer's hands, and the volunteer then redressed. The first three NC samples (1A, 1B & 1C) were then placed back into their sample tubes concluding the mock examination, no earlier than 30 min from the time these first NC samples were exposed to the environment.

Table 2
A list of sample types recovered and their function in this study.

Samples	Function
Thigh Swabs	To identify any potential contamination introduced to a forensic sample within the forensic medical examination room
Hand Swabs	To be analysed if required to eliminate as a potential source of contamination to the thigh sample
NC1 Samples (1A, 1B & 1C)	To identify airborne DNA in the environment throughout the duration of a forensic medical examination thereby providing the worst-case scenario for time of exposure.
NC2 Samples (2A, 2B & 2C)	To identify airborne DNA in the environment for the duration of time it takes to recover a forensic sample.
Environmental Monitoring Sample x6	To identify DNA present on the surfaces within the forensic medical examination room.
Eliminations Samples FHP, Custody Officer or Crisis Worker.	To be eliminated from DNA that might be detected on the NC samples or samples recovered from the volunteer.
Volunteer Elimination Samples	To be eliminated from the samples recovered from the volunteer, to determine contamination present.

The function of each sample type has been summarised in Table 2:

The collected moist and dry swabs were frozen and stored prior to transporting to Cellmark Forensic Services for DNA testing. All swabs were processed utilising ISO 17025 accredited processes for extraction, quantification and analysis using DNA-17 Next Generation Multiplex (NGM) Select. Results were provided as electrophoretograms, quantification (quant) scores and allele counts with individual allele peak height information.

2.2. Phase 2: assessment of the impact of air replacement rates on contamination risk and suitability of air sampling for environmental monitoring

Phase 2 testing involved four SARCs, where NC samples and an air sampler were placed into the forensic medical examination rooms throughout mock medical examinations and also on five separate occasions within each SARC between live cases (whilst the room was not in use).

A Coriolis Compact air sampler (Bertin Technologies) was utilised, which provided a 50 L/min airflow rate and a 0.5–10 μm particle size collection range. This device concentrated airborne particles by vortexing the sampled air into a tube that had previously undergone ethylene oxide treatment to ensure that it was free of any contaminating DNA. The tubes and the NC samples were then stored frozen prior to transporting to Cellmark Forensic Services for DNA testing, as per phase 1.

The four SARCs involved in phase 2 of the study are detailed in Table 1. At each SARC, following a routine clean of the forensic medical examination room, three moistened NC swabs were placed upright in pots with the tip facing upwards, in the following locations:

- Examination couch
- Sampling trolley
- Work surface

The air sampler was placed in close proximity to the examination couch. First, a process negative control (PNC) sample was taken by placing a tube into the sampler and locking it in, before removing it, placing the lid on and bagging it. This process was repeated with a second sampling tube, which was then left locked in and the air vacuum system switched on.

The purpose of the PNC air sample was to eliminate any potential DNA introduced through the process carried out by the operator that might otherwise have been introduced to air samples that were subsequently taken.

The NC swabs and the air vacuum system (switched on) remained in situ up until the point of use of the room for a forensic medical

examination (timescales ranged from 45 min to 8 h 15 min). Prior to a patient entering the medical examination room the three negative control samples were collected (replaced into their sampling tube and all sample tubes placed into one evidence bag) and the air sampling tube removed from the sampler, the lid placed back on and placed into an evidence bag.

This process was repeated five times at each of the four SARCs, in total providing 60 NC samples and 20 air flow samples (and 20 PNC air flow samples).

In addition, a mock medical examination that replicated the process followed in phase 1, but with the additional use of the air sampler, was conducted in SARC (L09) with >20 whole room replacement per hour airflow and SARC (L06) without an airflow system. The purpose of these experiments was to identify any differences in the level of DNA in the air whilst a forensic medical examination was underway.

2.3. Coriolis Suitability Testing

Some additional tests were conducted as positive controls to assess the overall effectiveness of the Coriolis instrument for collecting human DNA from the air by testing environments within which significant levels of airborne skin particles might reasonably be expected. Air samples were collected from a) a busy public cocktail bar, b) a public toilet and c) the living room of a family house with 3 inhabitants. EM swabs were also collected from three areas within each location.

2.4. Statistical methods

A students t-test was used to statistically analyse data from phase 1 and 2. The ratio of the difference in the group means, for the data sets listed below, over the pooled standard error of both groups were used to determine if the differences were statistically significant.

i. Custody EM results compared with SARC EM results,
ii. Facilities with the highest and lowest EM results,
iii. NC 1s compared with NC 2s,
iv. Air sample results from SARC compared with custody
v. SARCs with >20 whole room replacement air flow compared with SARCs with <20.

The following formula was used to determine whether the difference in the group means were significant or not using a 95 % confidence interval.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)\right)}}$$

3. Results

3.1. Phase 1

a) Assessment of environmental DNA contamination levels

The total number EM samples taken from each high contamination risk area prior to the mock forensic medical examinations are shown in Appendix 1B. DNA was identified in 121 of the 144 EM samples (84 %), the DNA present in 92 of these samples was sufficient for comparison purposes. EM results for each sampling event are provided in Appendix 1A. The lowest, highest and average allele counts for each EM location are recorded in Appendix 1B, with average allele counts shown in Fig. 1.

In total, 72 EM samples recovered from SARCs were analysed and gave a range of 0–47 alleles with an average of 10 alleles detected per sample. 72 EM samples recovered from custody suites gave a range of 0–114 alleles with an average of 52 alleles per sample. As shown in Appendix 1C t-tests of equal variance and of unequal variance showed that there is a significant difference between the results from SARC and custody environments, indicating that the overall background DNA levels within the sampled custody facilities were significantly higher than in SARCs.

Large differences in contamination levels were observed between different custody suites. Comparison of custody L03 and L02 with the lowest and highest total numbers of alleles identified from their 18 EM results, respectively, is shown in Appendix 1D: t-tests of equal variance and of unequal variance demonstrated a statistically significant difference between the results. Similarly, the same comparison conducted between the SARCs with the lowest (L08) and highest (L06) total number of observed alleles also showed contamination levels to be statistically significantly different (Appendix 1E).

EM results are summarised in Appendix 2 and displayed graphically in Fig. 2.

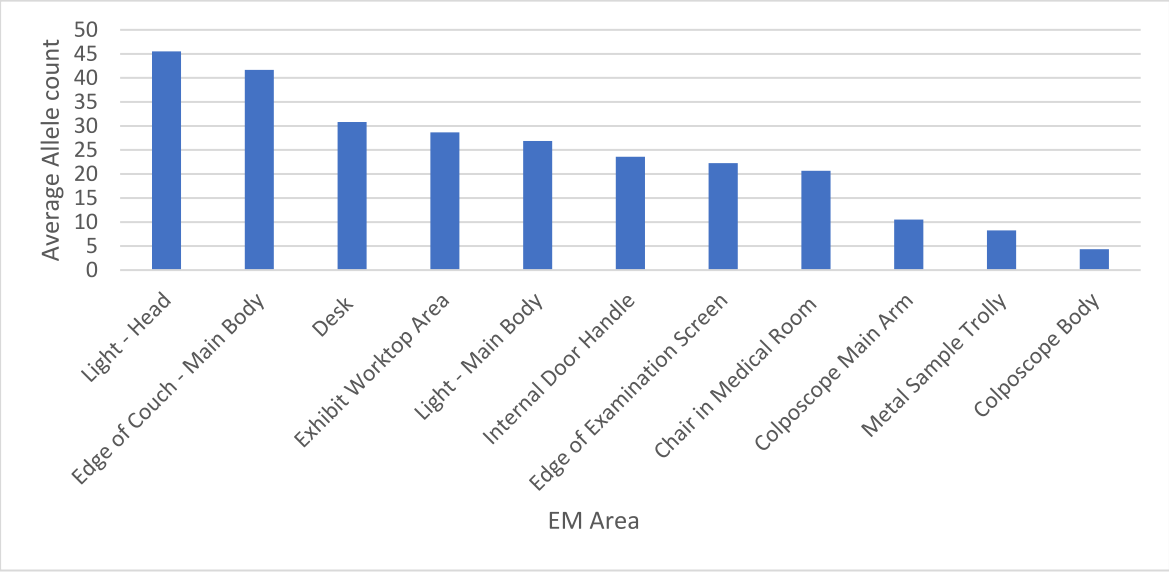

Fig. 1. A graph to show the average allele count from the 144 EM samples recovered from each of the 11 different sampling points within the forensic medical examination rooms of the 4 SARCs and 4 custody suites.

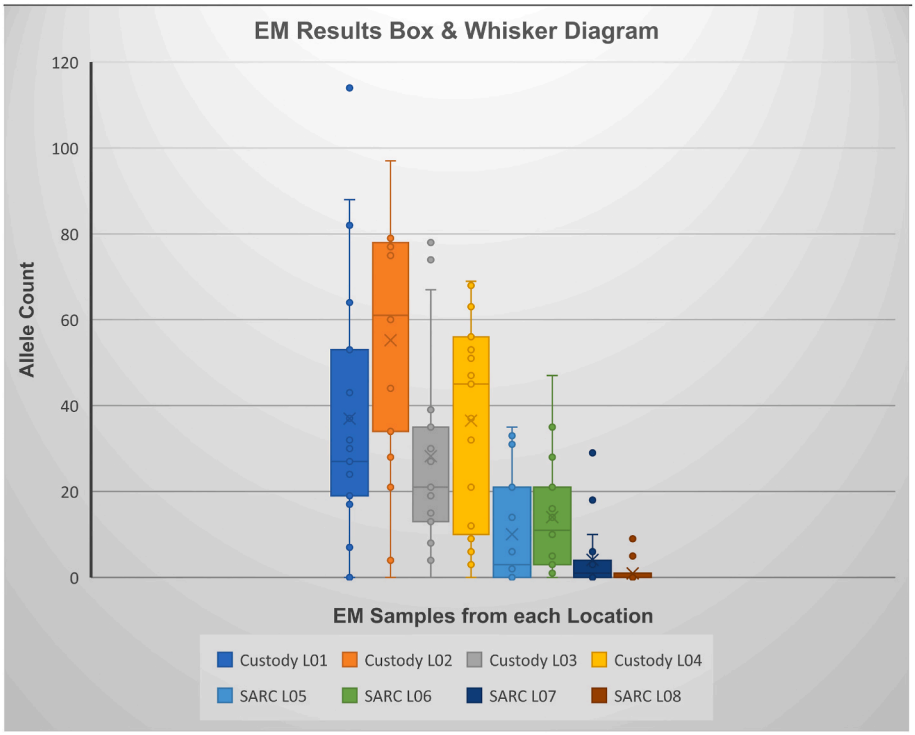

Fig. 2. A box and whisker diagram summarising the EM results (as allele count) displaying the minimum, first quartile, median, third quartile, and maximum allele count for the 18 EM samples recovered from each of the four SARCs and four custody forensic medical examination rooms.

b) Assessment of risk to evidential samples from airborne contamination

NC swabs, together with mock casework samples were taken in order to identify the potential contamination risk from airborne DNA within the forensic medical examination rooms during the mock medical examination.

71 NC samples were tested which were exposed to the air during the entirety of the medical examination ranging in duration from 30 to 64 min with an average of 37 min (NC1), Appendix 3.

72 NC samples were also taken that were exposed for the duration of the swabbing part of the examination only, ranging from 1 to 7 min, average of 3 min (NC2), Appendix 4.

The results from both NC1 and NC2 samples have been summarised in Fig. 3.

The results show that 140 out of the total 143 NC samples yielded

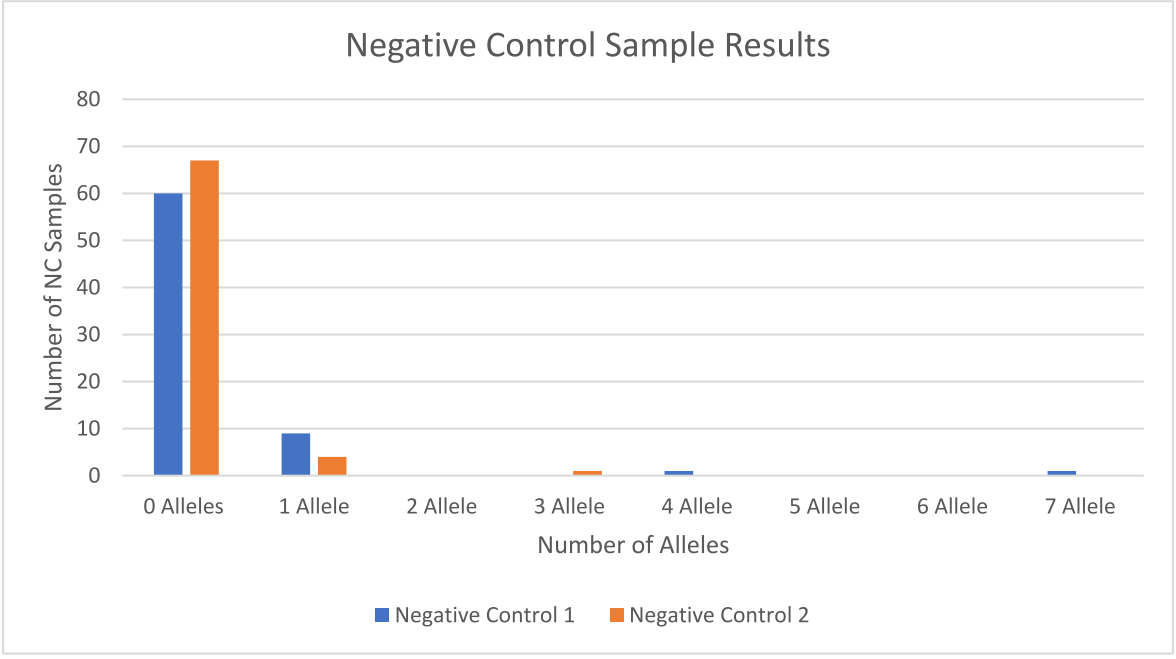

Fig. 3. A graph of the NC1 allele count results (30–64 min exposure to the forensic medical examination room environment) & NC2 (1–7 min exposure to the forensic medical examination room environment).

either zero or 1 allele result. 3 outlier results detecting 7, 4 and 3 alleles were subject to re-PCR, yielding 3, 1 and 1 alleles respectively on re-testing, and therefore no contamination results were sufficient for comparison purposes. On average 0.1888 alleles per sample (0.1258 alleles per sample if based on repeat PCR results) have been identified on the 143 NC samples tested. This data demonstrates that despite 7 out of the 8 medical examination facilities not meeting the >20 times whole room replacement per hour airflow requirement, negligible risk was demonstrated to the forensic samples becoming contaminated from airborne DNA in the forensic medical examination rooms.

The results for the initial NC sample sets (1-A, 1-B, 1-C) and subsequent sample sets (2-A, 2-B, 2-C) for custody have been compared against those same sets within SARCs, as shown in Appendix 5A & 5B. T-tests of equal variance showed that there was no significant difference in these results.

The 72 NC1 samples have been compared against the NC2 samples, as shown in Appendix 5C. T-tests of equal variance found that there is no significance difference in the results. This test has demonstrated that there is no significant difference in risk of contamination from the air between exposure times of 30–64 min and 1–7 min.

Of the 24 mock casework samples (thigh swabs) that were recovered from volunteers, 20 recovered DNA matching only the volunteers DNA, with no remaining components suitable for DNA comparison. In the remaining four thigh samples, a match to the volunteer's DNA could not be determined because insufficient DNA had been recovered for this comparison. This is common for skin samples recovered in SARCs as the aim of the sampling is to recover foreign DNA from the surface of the patient's skin, therefore the technique is designed to minimise recovery of the patient's own DNA. Results are shown in Appendix 6.

In all 24 thigh swabs, no DNA other than the volunteers' DNA had been recovered, evidencing in each case, that there has been no contamination introduced to the thigh swab within any of the forensic medical room environments tested.

Hand swabs were taken from the volunteer as a precaution. If DNA had been identified on the volunteer's thigh swabs which did not match the volunteer's DNA, then hand samples were available to support the investigation into the source of the contamination. Although this further testing ultimately was not required for this purpose, one of the wet and dry hand swabs from each mock examination was tested for completeness.

21 of the 24 hand swabs contained DNA that matched only the volunteers DNA. Two samples did not contain sufficient DNA for a comparison. One of the samples contained DNA that matched the volunteer, and DNA from an additional contributor suitable for comparison. The table of results for the hand swabs is shown in Appendix 7.

3.2. Results phase 2

a) Impact of airflow on contamination

3.3. Air sampling throughout a mock forensic medical examination

The results from the two mock medical examinations are provided in Appendix 8. The levels of DNA identified from the EM samples ranged from 1 to 29 alleles in SARC L09 with the >20 whole room replacement per hour, and 14 to 62 alleles in the SARC L06 without airflow. Fig. 4 shows the EM results as both quant scores and allele count and Fig. 5 (allele count) and Fig. 6 (quant score) provides a box and whisker diagram of these results.

No significant levels of DNA were identified in the NC samples from both SARCs, with 0 alleles in 11/12 NC samples and 1 allele in 1 NC sample.

Only the volunteer patient's DNA was identified on the thigh swabs recovered, demonstrating there has been no contamination introduced to the evidential sample within the medical room environments with or without the airflow required.

The air vacuum sample from the SARC L09 had 1 allele present and SARC L06 had 13 alleles present, all of which matched the operator's DNA.

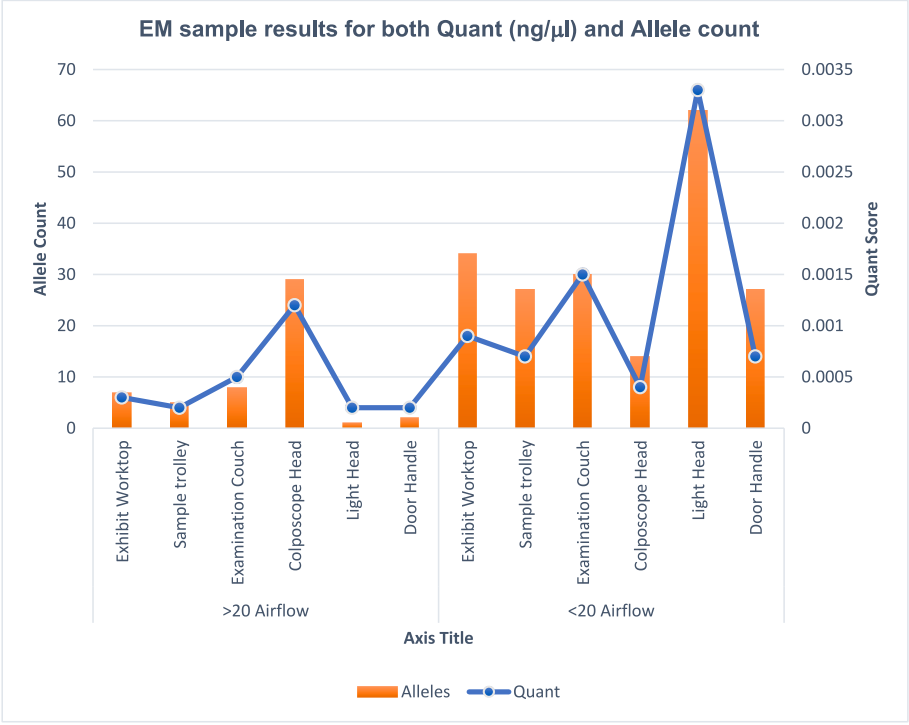

Fig. 4. A graph of the EM sample results shown as allele count and quant scores (ng/µl) for each of the 6 high risk areas of the forensic medical examination rooms. Results are shown for the 2 SARCs with a >20 whole room replacement airflow systems and 2 SARCs with <20 whole room replacement airflow systems in phase 2.

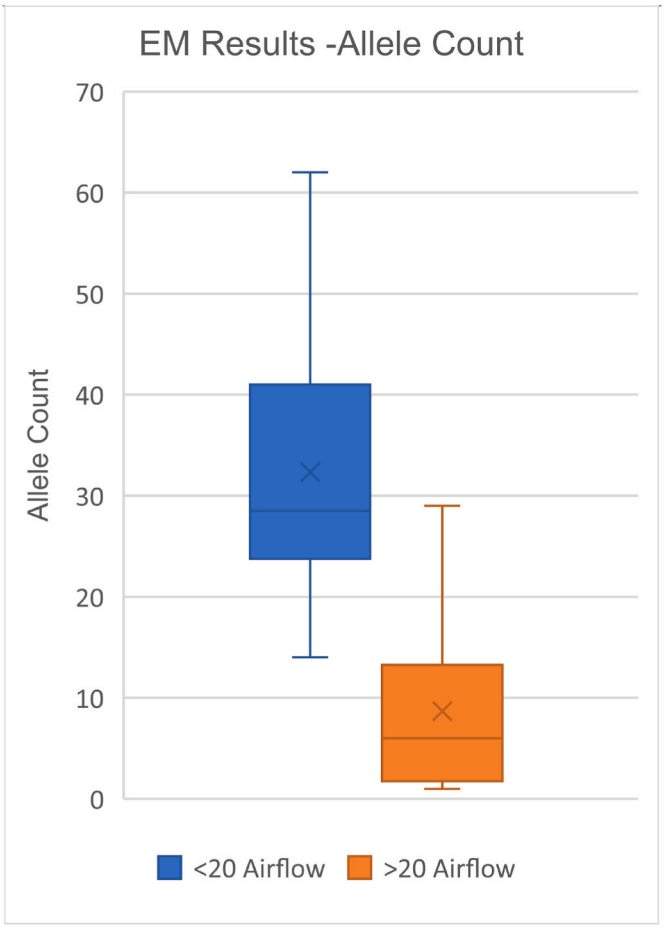

Fig. 5. A Box & whisker diagram of phase 2 EM results for the 2 SARCs with a >20 whole room replacement airflow systems and 2 SARCs with <20 whole room replacement airflow systems. Results shown as allele count.

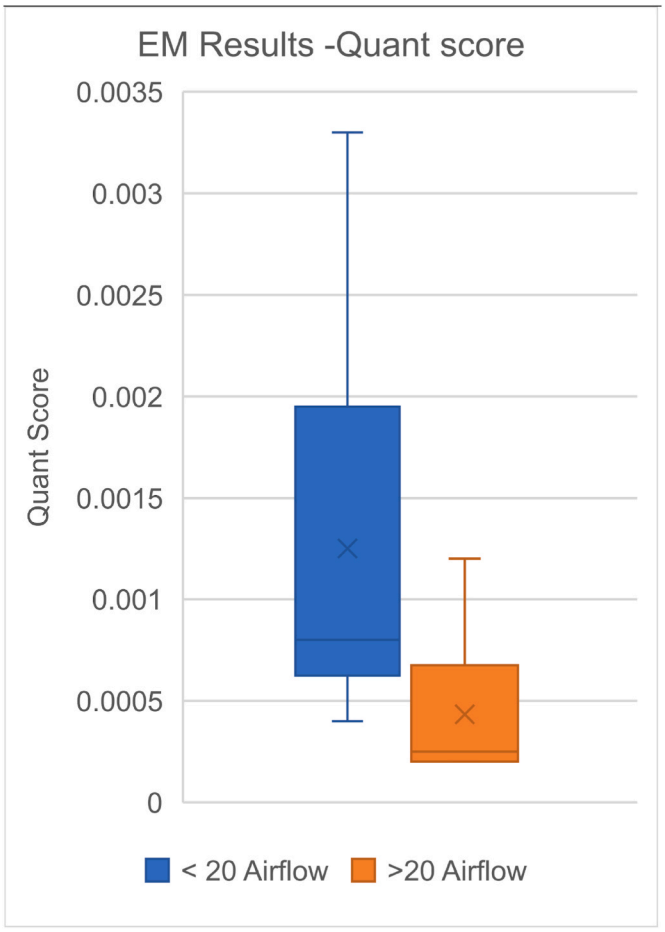

Fig. 6. A Box & whisker diagram of phase 2 EM results for the 2 SARCs with a >20 whole room replacement airflow systems and 2 SARCs with <20 whole room replacement airflow systems. Results shown as quant score (ng/µl).

3.4. Air sampling between forensic medical examinations

The time duration the NC samples were exposed and air sampled ranged from 1 to 28 h. The NC results from the 20 tests conducted at four SARCs (L09, L05, L06 and L10) are shown in Appendix 9 and summarised in Fig. 7. 52/60 NC samples had 0 alleles, and six NC samples had 1 allele. Only two NC samples had DNA suitable for comparison with 11 and 8 alleles, both of which were from SARC L09 which has an airflow of >20 whole room replacement per hour.

All results from the air vacuum samples found no levels of DNA suitable for comparison, results are shown in Appendix 10 and Fig. 7.

3.5. Coriolis Suitability Testing results

Results from the Coriolis Suitability Testing are shown in Appendix 12 and summarised in Table 3. The air sampling time within these environments ranged from 1 to 28 h.

4. Discussion

4.1. Study limitations

This data set only represents a small number of SARC and custody facilities. At the time of this study there were 54 SARCs in service across England and Wales, 4 of these (7 %) participated in phase 1 and 2 (4 %) in phase 2 and only 4 (2 %) out of approximately 210 custody suites participated in both phases 1 and 2 of this study.

The forensic medical examinations, although mimicking real life case scenarios, were performed on volunteer patients who were explicitly compliant. This is not representative of human behaviour where suspects in custody may not be so complaisant, and victims can be in trauma.

There is no published research on the suitability of testing air for DNA using the Coriolis air sampler. This is the first study to consider its ability to capture human DNA for subsequent DNA analysis.

4.1.1. Phase 1

Contamination hot spots identified within the forensic medical examination rooms correlate to a previous study[12] therefore, the areas sampled in this study provided worst case scenario by only targeting the areas of highest risk.

This study has highlighted significant variances in background DNA levels both within and between SARCs and custody suites as demonstrated in Appendix 1. Significantly higher levels of DNA were identified in custodies in comparison to SARCs. This is likely due to the custody medical examination rooms in this study having dual functionality, being used for both forensic medical examinations where potential critical evidence was recovered as well as an office for medical staff where medical examinations and discussions, unrelated to forensics occurred. Furthermore, although routine cleaning processes were in place in custody, these were not as frequent, nor as thorough as those employed in the SARCs.

SARC L08 displayed significantly lower background levels of DNA than the other 3 SARC facilities and provides an excellent learning opportunity to promulgate best practice within the forensic medical examination field. A fundamental difference is that SARC L08 was the only

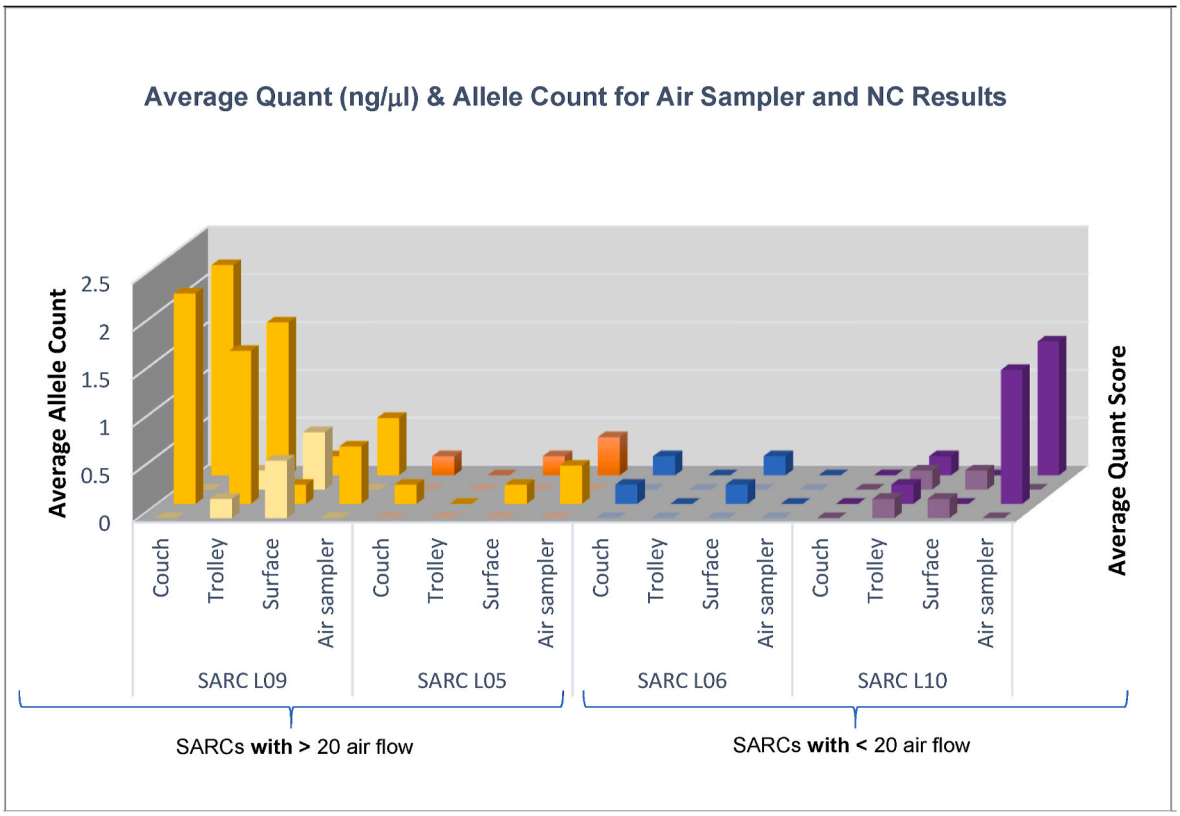

Fig. 7. A bar graph to show the average quant (ng/µl) and allele count results for phase 2 negative control samples & air samples for each of the 4 SARCs in phase 2.

Table 3
A table or air sampling and EM results for the 5 different locations tested, with the levels of human traffic and cleaning regime for each site, providing a comparison of DNA recovery from air sampling versus surface swabbing from 2 exemplar SARCs compared with other indoor environments.

Location	Human Traffic Levels & Cleaning regime	Air sampling			Surface swabbing (EM)		
		Av. No. alleles	Av. Conc. ng/µl	Median yield (ng)	Av. No. alleles	Av. Conc. ng/µl	Median yield (ng)
SARC >20 air change/hr L09	Low traffic/High cleaning	1	0	0	9	0.00043	0.0125
SARC <20 air change/hr L06	Low traffic/High cleaning	13[a]	n/a	n/a	32	0.00125	0.040
Domestic house, with 3 occupants	Low traffic/Low cleaning	0	0.0003	0.015	37	0.0066	0.270
Public toilet	High traffic/Low cleaning	58	0.007	0.35	55	0.0083	0.23
Public cocktail bar	V. high traffic/Low cleaning	125	0.224	11.20	115	0.0402	2.43

[a] Experimental contaminant: profile matched operator.

facility to have introduced monthly EM testing from which lessons had been learned and corrective actions taken to improve the cleaning process. Additionally, it was the only facility to be decontaminating surfaces using Actichlor, which is a bleach-based cleaning reagent, and is therefore, highly effective for DNA contamination removal.

Three FSPs in the UK offer an EM service to SARCs with each applying different criteria and advice. By way of an example, the most stringent of these FSP criteria categorises EM results of one or more alleles >400rfu, or more than 7 alleles at any peak height as a fail, with a recommendation to temporarily close the facility, re-clean and re-take EM samples. If these criteria are applied to the EM results in this study, 53 out of the 72 (74 %) EM samples from custody failed and 10 out of 72 (14 %) of EM samples from SARCs also failed. Every set of EM samples recovered prior to each of the 12 mock examinations in custody would include a failed result and for SARCs, 4 out of the 12 sets of EM samples would include a failed result, totalling 16 closure recommendations of the forensic medical examination rooms awaiting a subsequent 'acceptable' result before being able to reopen. Based on these results, if this EM criteria were to be routinely used by SARCs to assess the cleanliness of forensic medical examination rooms, then 100 % of custody suites and 33 % of SARCs would be out of use following each round of EM testing, which would undoubtedly have a significant detrimental effect to these services and an even greater impact to victims. Here lies the issue with victim/public safety-led services aspiring to meet DNA laboratory levels of cleanliness, which is not practical or necessary as this standard is not proportionate to the risk. The majority of the EM results from this study highlight the requirement for more effective cleaning within the forensic medical examination rooms and the need for cleaning processes and products to undergo validation/verification, as required by the FSR Code of Practice[8] to demonstrate they are fit for purpose.

Despite very high environmental DNA contamination levels observed in some of the environments surveyed, contamination was not apparent in the thigh swabs that mimicked evidential recovery. This indicates that other anti-contamination practices in place (e.g. PPE, Standard Operating Procedures (SOP)s, use of forensic DNA grade consumables etc) effectively manage the contamination risk presented to evidential recovery undertaken in an environment with high levels of background

DNA. The risk, therefore, of background DNA contaminating the forensic evidence recovered within these facilities is low. That is not to say that effective cleaning is unimportant, but rather it is one of a raft of anti-contamination measures that, in combination, minimise the risk of DNA contamination. A clean environment is especially important should one of the other measures be inadvertently compromised, such as failure to change gloves after a surface has been accidently touched. Therefore, every effort should be made to achieve and maintain a clean working environment.

The risk of DNA contamination from the air was demonstrated to be low based on the NC swab results. A consideration in the development of this set of experiments was to assess whether this type of control might be of use in routine environmental monitoring. Detected levels of DNA were largely negligible, with the exception of one outlier result of 7 alleles that could not be replicated on re-PCR) 140/143 of the negative controls yielded zero or 1 allele, which by way of context, this is the level of DNA deemed acceptable when batch testing DNA consumables.[25] For the remaining 3/142 controls, none of the additional alleles were detectable in the corresponding thigh swab. These results demonstrate that there is little to no DNA in the air within SARC and custody forensic medical examination rooms despite high levels being present on surfaces.

4.1.2. Phase 2

The NC results from phase 1 conducted in four SARCs and four custodies with no air flow replacement systems, have demonstrated that there were no significant levels of DNA in the air within the forensic medical examination rooms. These results demonstrate that there was no risk of contamination from the air to a swab used to recover forensic samples in SARCs that have no airflow replacement systems.

Air vacuum samples taken in the forensic medical examination room during mock medical examinations on volunteer patients, and air samples taken whilst the room was not in use, all showed no greater than 4 alleles present. None of the air vacuum samples had levels of DNA suitable for comparison, with the exception of the sample contaminated by the operator.

A *t*-test (Appendix 11A & 11B) has been conducted on the allele count and quant scores for the air vacuum samples which has demonstrated that there is no significant difference between the levels of DNA found in the air in SARCs with or without the >20 whole room replacement per hour airflow.

Therefore, no evidence has been found that demonstrates there is less DNA present in SARC forensic medical examination rooms with a >20 whole room replacement per hour airflow when compared to SARCs with ~10 whole room replacement per hour, or SARCs with no airflow system.

Greater levels of DNA were found on NC samples taken in a SARC with >20 whole room replacement air flow, which further demonstrates that increased air flow does not decrease the levels of DNA in the air.

4.2. Suitability of air sampling for environmental monitoring

The results in Table 3 demonstrate that in busy public indoor spaces (bar and toilet) with a high degree of people "traffic" and concomitant high air disturbance, the measured DNA levels using air sampling and surface swabs were both high and broadly equivalent. In contrast, sampling from low air disturbance areas, gave higher levels of detectable DNA by swabbing compared with air sampling, irrespective of whether this was a regularly cleaned area, such as a SARC examination suite, or from an irregularly cleaned domestic living area. These results are from a very small sample size but correlate well with findings from a larger study in which offices, meetings rooms and DNA laboratories were sampled.[18] It also confirms that swabbing of surfaces provides a more sensitive environmental sampling technique than air sampling for low air disturbance areas such as SARCs and custody suites.

In some instances, a greater number of alleles (and higher quant values) were observed in custody EM samples when compared to those recovered from a public toilet or a private residence, however, no DNA was detected from the air samples recovered from these medical examination rooms. A recent study [27] investigating the level of DNA individuals transfer to untouched items or surroundings found that even if surfaces and/or objects were not directly contacted this transfer can occur. Distance from a person, the length of time and the person themselves all play a role in the quantity of DNA that is deposited to one's surroundings. The high EM results and negligible air sampling results found in custody may be attributed to lower air movement combined with the use of PPE and lower levels of human traffic which could potentially re-aerosolise settled DNA, significantly reducing the amount of DNA present in the air within these controlled environments.

5. Conclusions

The results from phase 1 of this study have shown that despite the detection of DNA in the SARC and custody forensic medical examination rooms at varying levels (including levels of DNA which would be classed as a failure based on current DNA laboratory standards), none of the DNA found in these environments has been identified on the forensic samples recovered.

The fact that high background levels of contamination did not adversely affect the evidential samples indicates that the real-life risk from environmental contamination is low and that other measures in place, including appropriate use of PPE and, following appropriate SOPs, are effectively managing the risk that background contamination presents. This justifies continuing to provide a forensic medical service despite laboratory deemed 'high' EM results. This study has demonstrated that applying DNA laboratory EM standards to a SARC or custody environment is not fit for purpose. This is not to disparage the value of cleaning these environments, all reasonable efforts should be taken to minimise background levels of contamination, recognising that this also reduces the risk of casework being compromised by inadvertent and indirect transfer e.g. on the gloves of the FHP if correct anti-contamination protocols are not followed. The use of EM is essentially a secondary rather than primary anti-contamination measure, as it is highlighting an indirect risk to casework that is only a potential factor if other safeguards in place failed significantly. This is in direct contrast with a primary anti-contamination measure of using only forensic DNA grade consumables for the recovery of DNA evidence, as failure to do so presents a high standalone risk of casework becoming compromised.

This study has identified opportunities for improvement in the background DNA levels of forensic medical examination rooms and supports FSR[8] requirements for the validation of cleaning process. Where EM can be used as an initial assessment of the ongoing efficiency of cleaning and this data used to justify a reduction in EM frequency where, over time, the contamination risk is deemed low. Results from this study provide a baseline to work from to demonstrate continuous improvement in the effectiveness of the cleaning processes deployed.

This study has been shared with the FSR where it was agreed that it provides sufficient data to change the FSR requirements on EM sampling and an agreed criteria, developed based on the data from this study, has been incorporated into the FSR SARC and custody guidance[1] as listed in Appendix 13. Whereby, for example, a red status result based on the DNA laboratory EM criteria would have previously required temporary closure, a red status using the new SARC-specific criteria requires a forensic clean of the affected area followed by EM sampling, which is a more proportionate response to the real-life risk posed.

The practical impact of utilising the FSR SARC EM criteria is illustrated in Table 4 by comparing these and those of the exemplar FSP risk levels applied to the EM data from both the best performing (SARC L08) and worst performing (Custody L02) facilities with respect to cleanliness.

The SARC-specific EM criteria accepts higher levels of DNA in the environment, eliminating the 'pass/fail' criteria and providing guidance

Table 4
A table of the average EM results (provided as allele count and quant score (ng/μl)) for each of the high-risk areas within the forensic medical examination rooms of SARC L08 and Custody L02. These results have been assessed against an FSP laboratory EM criteria and the SARC-specific criteria developed from the data in this study (shown in Appendix 13).

	SARC L08				Custody L02			
	Average Allele	Average Quant score	FSP Criteria	FSR SARC EM Criteria	Average Allele	Average Quant score	FSP Criteria	FSR SARC EM Criteria
Desk	24.5	0.0011	Red	Amber	78.3	0.0150	Red	Red
Colposcope Body	10.7	0.0007	Red	Amber	N/A	N/A	N/A	N/A
Edge of Examination Screen	7.5	0.0002	Red	Green	25.3	0.0015	Red	Amber
Metal Sample Trolly	29.0	0.0021	Red	Amber	N/A	N/A	N/A	N/A
Internal Door Handle	3.0	0.0002	Pass	Green	56.7	0.0174	Red	Red
Light - Head	22.0	0.0019	Red	Amber	61.0	0.0000	Red	Red
Colposcope Main Arm	10.5	0.0004	Red	Amber	N/A	N/A	N/A	N/A
Edge of Couch - Main Body	2.0	0.0000	Pass	Green	66.0	0.0055	Red	Red
Exhibit Worktop Area	35.0	0.0016	Red	Red	84.3	0.0097	Red	Red
Chair in Medical Room	17.0	0.0019	Red	Amber	28.0	0.0014	Red	Amber
Light - Main Body	10.0	0.0002	Red	Green	N/A	N/A	N/A	N/A

of what actions to take based on different levels of DNA identified in the environment, shown in Appendix 13. The revised criteria has been adopted by FSPs across England and Wales, standardising the approach to SARC and custody EM nationally.

This study has led to a national change in the way in which SARC and custody EM results are evaluated, ensuring that temporary facility closures are no longer required, thereby lessening the negative impact this would have on a victim and reducing the demand on police resources. In addition, reducing resource time in investigating EM failures and the likelihood of holding up a case in the CJS, which is a welcoming change following the most recent crime in England and Wales report where police recorded sexual offences rose by 32 % to the highest annual figure recorded in England and Wales (194,683 offences). This included the highest recorded annual number of rape offences to date (70,330 offences).[3]

Phase 2 of this study concludes that >20 whole room replacement per hour airflow does not reduce the level of DNA in the air compared to forensic medical examination rooms with <20 whole room replacement or no airflow. There was no measurable benefit, albeit this was determined from a small sample size. Based on the data from this study the FSR has updated the recommendations in the SARC and custody guidance to remove this requirement as an anti-contamination measure. Best practice in the updated guidelines now recommends 10 whole air replacements, in line with recommendations for medical general treatment facilities.[26]

Assessment of results from SARCs comparing DNA recovery by air sampling versus swabbing surfaces demonstrated the latter generates significantly higher yields in these low people traffic and highly cleaned environments, making it easier to monitor levels and the impact of change to them. This lower sensitivity combined with the longer sampling time required makes air sampling less convenient as an alternative EM approach for forensic medical examination facilities.

6. Next steps

Implementation of the findings from this study within the updated FSR Code of Practice[8] will save significant costs through both realistic targets for air flow management systems, and reduced DNA testing costs from more achievable EM target levels. It also gives an overall better understanding of the risks environmental DNA contamination pose. This in turn has led to responses that are more proportionate to the real-life risks and that, for example, no longer stipulate closure of a facility purely on the grounds of detected EM levels due to other anti-contamination measures in place effectively manage the contamination risk.

This study has highlighted a need for improvement in the forensic anticontamination measures in custody forensic medical examination rooms to ensure the same level of risk mitigation is taken during the examination of suspects as there is for complainants.

This research has concluded that there is scope to improve the cleaning processes to reduce the levels of detectable DNA in the environment through method development and the validation of cleaning processes.

Informed participant consent

The project lead met with the participants to obtain informed consent for enrolment into this study. During this enrolment, the informed consent was discussed in its' entirety and the participants were given the opportunity to ask questions, and all questions were answered. The participants signed and dated study informed consent forms.

CRediT authorship contribution statement

Michelle Gaskell: Project Management, Conceptualisation, Methodology, Investigation, Funding acquisition, Data curation, Writing – original draft, Writing – review and editing. Kevin Sullivan: Conceptualisation, Methodology, Writing -review and editing. June Guiness: Conceptualisation, Methodology, review and editing.

Ethics approval

The research plans for this study were reviewed by the Home Office Biometric and Forensic Ethics Group prior to commencing the study and all recommendations raised had been addressed prior to commencement.

No SARC patients were involved in this study. All volunteers that participated in this study provided written consent prior to involvement. The mock cases were carried out in accordance with ''The Code of Ethics ' of the World Medical Association.

Declarations of interest

The authors whose names are listed certify that they have no affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Appendix

Appendix 1A

Table 5
A table of the 6 EM Results for each of the 24 mock forensic medical examinations conducted at the four SARCs and four custody suites in Phase 1.

Location		EM Results					
Mock Exam Ref.	Location	EM 1 Alleles	EM 2 Alleles	EM 3 Alleles	EM 4 Alleles	EM 5 Alleles	EM 6 Alleles
ME01	Custody L01	24	7	20	43	88	37
ME02		27	53	0	17	20	82
ME03		30	64	25	32	19	114
ME04	Custody L02	77	28	97	76	44	21
ME05		79	44	78	60	75	61
ME06		79	4	78	34	79	35
ME07	Custody L03	31	19	21	27	74	19
ME08		39	30	67	4	15	28
ME09		9	78	19	13	35	8
ME10	Custody L04	51	47	57	45	37	54
ME11		68	10	21	69	56	63
ME12		32	3	12	9	6	53
ME13	SARC L05	33	0	1	35	0	31
ME14		21	0	3	2	6	0
ME15		14	31	7	7	0	0
ME16	SARC L06	28	10	1	11	3	22
ME17		5	21	5	14	3	2
ME18		16	17	47	35	17	10
ME19	SARC L07	1	0	1	18	29	1
ME20		10	3	0	0	0	1
ME21		1	4	6	1	1	1
ME22	SARC L08	0	0	0	0	1	0
ME23		0	0	0	0	1	1
ME24		5	0	1	0	0	9

Appendix 1B

Table 6
A summary table of the lowest, highest and average allele counts from the EM samples recovered from each of the high-risk areas of the 4 SARCs and 4 custody suites in phase 1, including the number of EM samples recovered from each of these areas within the forensic medical examination room.

No. of Samples	EM Location	Lowest Alleles Count	Highest Allele Count	Average Allele Count
6	Light - Head	1	82	46
12	Edge of Couch - Main Body	0	88	42
21	Desk	0	79	31
20	Exhibit Worktop Area	0	97	29
7	Light - Main Body	0	114	27
20	Internal Door Handle	0	69	24
19	Edge of Examination Screen	0	78	22
12	Chair in Medical Room	0	63	21
10	Colposcope Main Arm	0	33	11
8	Metal Sample Trolley	0	47	8
9	Colposcope Body	0	17	4

Appendix 1C

Table 7
The *t*-test calculations of the 72 EM (allele count) results from 4 custody suits compared to the 72 EM (allele count) results from 4 SARCs in phase 1, determining that these results are significantly different.

Statistics	Custody EM Results	SARC EM Results	
Average	41.389	7.694	
Standard Deviation	27.010	11.113	
Variance	729.537	123.511	
Count	72	72	
Coefficient of Variation	65.259	144.436	
Confidence	0.95	0.05	
F-Test			
F-Calculated	0	F table	19
***t*-Test Equal Variances**			
Spool	3.466		
t Calculated	99.906		
t table	1.978	**The difference is significant**	
***t*-Test unequal variances**			
DF	94.371		
t calculated	9.789		
t table	1.986	**The difference is significant**	

Appendix 1D

Table 8
The *t*-test calculations of the 18 EM (allele count) results from custody suite L02 (the facility with the highest EM results) compared to the 18 EM (allele count) results from SARC L03 (the facility with the lowest EM results) in phase 1.

Statistics	Custody L02 Highest EM Results	Custody L03 Lowest EM Results
Average	29.778	58.278
Standard Deviation	22.079	25.795
Variance	487.477	665.389
Count	18	18
Coefficient of Variation	74.146	44.262
Confidence	0.95	0.05
F-Test		

(*continued on next page*)

Table 8 (*continued*)

Statistics	Custody L02 Highest EM Results	Custody L03 Lowest EM Results
F-Calculated	0	
***t*-Test Equal Variances**		
Spool	8.235	
t Calculated	58.050	
t table	2.0322	**The difference is significant**
***t*-Test unequal variances**		
DF	33.209	
t calculated	3.5611	
t table	2.0345	**The difference is significant**

Appendix 1 E

Table 9
The *t*-test calculations of the 18 EM (allele count) results from SARC L06 (the SARC with the highest EM results) compared to the 18 EM (allele count) results from SARC L08 (the SARC with the lowest EM results) in phase 1.

Statistics	SARC L06 Highest EM Results	SARC L08 Lowest EM Results
Average	1	14.833
Standard Deviation	2.3263	12.382
Variance	5.412	153.324
Count	18	18
Coefficient of Variation	232.632	83.477
Confidence	0.95	0.05
F-Test		
F-Calculated	0	
***t*-Test Equal Variances**		
Spool	3.0557	
t Calculated	10.350	
t table	2.0322	**The difference is significant**
***t*-Test unequal variances**		
DF	18.199	
t calculated	4.659	
t table	2.101	**The difference is significant**

Appendix 2

Table 10
A table of EM statistics for the 18 EM results from each of the four custody suites and four SARCs, providing the minimum, 1st quartile, median, 2nd quartile and mean results.

Location	Minimum	1st Quartile	Median	2nd Quartile	Maximum	Mean
Custody L01	0	19.5	30	58.5	114	39.882
Custody L02	4	34.5	61	78.5	97	57.176
Custody L03	4	14	21	37	78	29.706
Custody L04	3	11	45	56.5	69	37.765
SARC L01	0	0	3	17.5	35	9.294
SARC L02	1	4	11	19	47	14.059
SARC L03	0	0.5	1	5	29	4.529
SARC L04	0	0	0	1	9	1.0588

Appendix 3

Table 11
The NC results, for phase 1, for sample 1's (1-A, 1-B, 1-C) which were exposed in the medical room for the duration of the forensic medical examination (30–64 min).

Mock Exam Ref.	Location Ref.	NC 1-A Alleles	Suitable for Comparison	NC 1-B Alleles	Suitable for Comparison	NC 1-C Alleles	Suitable for Comparison
ME01	L01	0	No	0	No	0	No
ME02		0	No	0	No	0	No
ME03		0	No	0	No	0	No
ME04	L02	0	No	0	No	0	No
ME05		0	No	0	No	7◆	CD
ME06		0	No	0	No	0	No
ME07	L03	0	No	0	No	*	*
ME08		0	No	1	No	1	No
ME09		0	No	0	No	0	No
ME10	L04	0	No	4▲	No	0	No
ME11		1	No	0	No	0	No
ME12		0	No	0	No	0	No
ME13	L05	0	No	1	No	0	No
ME14		1	No	0	No	0	No
ME15		0	No	0	No	0	No
ME16	L06	0	No	0	No	0	No
ME17		0	No	0	No	0	No
ME18		0	No	0	No	0	No
ME19	L07	0	No	0	No	0	No
ME20		0	No	0	No	0	No
ME21		1	No	0	No	1	No
ME22	L08	0	No	0	No	0	No
ME23		0	No	0	No	1	No
ME24		1	No	0	No	0	No

*Samples not suitable for testing due to FSP human error.
◆ME05 -NC1-C Initial test result was 7 alleles, repeat PCR test result was 3 alleles and the second repeat PCR result was 3 alleles.
▲ME10-NC1-B Initial test result was 4 alleles, repeat PCR test result was 1 allele and the second repeat PCR result was 0 alleles.

Appendix 4

Table 12
The NC results, for phase 1, for sample 2's (2-A, 2-B, 2-C) which were exposed in the forensic medical room for the duration of the sampling activity (1–7 min).

Mock Exam Ref.	Location Ref.	NC 2-A Alleles	Suitable for Comparison	NC 2-B Alleles	Suitable for Comparison	NC 2-C Alleles	Suitable for Comparison
ME01	L01	0	No	0	No	0	No
ME02		0	No	0	No	0	No
ME03		0	No	0	No	0	No
ME04	L02	0	No	0	No	0	No
ME05		0	No	0	No	0	No
ME06		0	No	0	No	0	No
ME07	L03	0	No	0	No	0	No
ME08		0	No	0	No	0	No
ME09		0	No	0	No	0	No
ME10	L04	0	No	0	No	0	No
ME11		0	No	0	No	0	No
ME12		0	No	0	No	0	No
ME13	L05	0	No	3*	No	0	No
ME14		0	No	1	No	0	No
ME15		0	No	0	No	0	No
ME16	L06	0	No	0	No	0	No
ME17		0	No	0	No	1	No
ME18		0	No	0	No	0	No
ME19	L07	0	No	0	No	0	No
ME20		0	No	0	No	1	No
ME21		0	No	0	No	0	No
ME22	L08	0	No	0	No	0	No
ME23		0	No	0	No	0	No
ME24		1	No	0	No	0	No

* ME13-NC2-B Initial test result was 3 alleles, repeat PCR test result was 1 allele.

Appendix 5A

Table 13
The *t*-test calculations of the custody NC1 sample (allele count) results compared to the SARC NC1 sample (allele count) results in phase 1.

Statistics	NC 1 Custody Results	NC 1 SARC Results
Average	0.4	0.167
Standard Deviation	1.355	0.378
Variance	1.835	0.143
Count	35	36
Coefficient of Variation	338.683	226.779
Confidence	0.95	0.05
F-Test		
F-Calculated	0	
***t*-Test Equal Variances**		
Spool	0.239	
t Calculated	0.355	
t table	1.995	**The difference is not significant**
***t*-Test unequal variances**		
DF	39.123	
t calculated	0.982	
t table	2.023	**The difference is not significant**

Appendix 5B

Table 14
The *t*-test calculations of the custody NC2 sample (allele count) results compared to the SARC NC2 sample (allele count) results in phase 1.

Statistics	NC 2 Custody Results	NC 2 SARC Results
Average	0	0.194
Standard Deviation	0	0.577
Variance	0	0.333
Count	36	36
Coefficient of Variation	Can not be calculated	296.569
Confidence	0.95	0.05
F-Test		
F-Calculated	0	
***t*-Test Equal Variances**		
Spool	0.097	
t Calculated	0	
t table	1.994	**The difference is not significant**
***t*-Test unequal variances**		
DF	35	
t calculated	2.023	
t table	2.030	**The difference is not significant**

Appendix 5C

Table 15
The *t*-test calculations of all the NC1 sample (allele count) results compared to all the NC2 sample (allele count) results in phase 1.

Statistics	NC 1 Results	NC 2 Results
Average	0.282	0.0972
Standard Deviation	0.988	0.417
Variance	0.977	0.174
Count	71	72
Coefficient of Variation	350.833	428.451
Confidence	0.95	0.05
F-Test		
F-Calculated	0	
***t*-Test Equal Variances**		
Spool	0.128	
t Calculated	0.554	
t table	1.977	**The difference is not significant**
***t*-Test unequal variances**		
DF	93.836	
t calculated	1.451	
t table	1.986	**The difference is not significant**

Appendix 6

Table 16
Thigh swab results (as allele count) from phase 1, recovered from the 24 mock forensic medical examinations. Including the results from the comparison of the DNA identified in each thigh swab to the volunteer patient and how many of those alleles could not be attributed to the volunteer.

Thigh Swab				
Mock Examination Ref.	Location Ref.	Thigh Swab Alleles	Comparison to Volunteer Elimination	Alleles not Attributed to the Volunteer
ME01	L01	31	Match	None
ME02		5	Match	None
ME03		18	Match	None
ME04	L02	8	Match	None
ME05		21	Match	None
ME06		15	Match	None
ME07	L03	31	Match	None
ME08		31	Match	None
ME09		1	CD	None
ME10	L04	30	Match	None
ME11		10	Match	None
ME12		2	CD	None
ME13	L05	33	Match	None
ME14		8	CD*	None
ME15		0	CD	None
ME16	L06	7	Match	None
ME17		14	Match	None
ME18		5	Match	None
ME19	L07	5	Match	None
ME20		27	Match	None
ME21		14	Match	None
ME22	L08	23	Match	None
ME23		32	Match	CD
ME24		36	Match	CD

*Following a RPCR the DNA identified on ME14 thigh Swab matched the volunteer.
CD= Cannot determine as the level of DNA present is not suitable for a comparison.

Appendix 7

Table 17
Hand swab results (as allele count) from phase 1, recovered from the 24 mock forensic medical examinations. Including the results from the comparison of the DNA identified in each hand swab to the volunteer patient and how many additional alleles detected that could not be attributed to the volunteer.

Volunteer Hand Swabs Results				
Mock Examination Ref.	Location Ref.	Right Hand Swab Alleles	Comparison to Volunteer Elimination	Remaining Components Suitable for Comparison?
ME01	L01	30	Match	None
ME02		4	Match	None
ME03		32	Match	None
ME04	L02	31	Match	None
ME05		31	Match	None
ME06		10	Match	None
ME07	L03	33	Match	None
ME08		32	Match	None
ME09		7	CD	CD
ME10	L04	22	Match	None
ME11		32	Match	None
ME12		5	CD	CD
ME13	L05	24	Match	None
ME14		23	Match	None
ME15		23	Match	None
ME16	L06	16	Match	None
ME17		15	CD	Yes
ME18		38	Match	CD
ME19	L07	27	Match	None
ME20		24	Match	None
ME21		31	Match	None
ME22	L08	8	Match	None
ME23		17	Match	None
ME24		40	Match	Yes

CD= Cannot determine as the level of DNA present is not suitable for a comparison.

Appendix 8

Table 18
Phase 2 mock medical examination EM, NC, thigh swabs and air vacuum sample results for SARC L09 and L06.

Location	Mock Examination Ref.	SARC L09 > 20 Airflow	SARC L06 < 20 Airflow
Environmental Monitoring Samples	**EM 1 Location**	Exhibit Worktop	
	EM 1 Alleles	7	34
	EM 1 Quant	0.0003	0.0009
	EM 2 Location	Sample Trolley	
	EM 2 Alleles	5	27
	EM 2 Quant	0.0002	0.0007
	EM 3 Location	Examination Couch	
	EM 3 Alleles	8	30
	EM 3 Quant	0.0005	0.0015
	EM 4 Location	Colposcope Head	
	EM 4 Alleles	29	14
	EM 4 Quant	0.0012	0.0004
	EM 5 Location	Light Head	
	EM 5 Alleles	1	62
	EM 5 Quant	0.0002	0.0033
	EM 6 Location	Door Handle	
	EM 6 Alleles	2	27
	EM 6 Quant	0.0002	0.0007
NC for duration of Mock Examination	**NC 1A - Alleles**	0	1
	NC 1A - Quant	0.0002	0
	NC 1B- Alleles	0	0
	NC 1B- Quant	0	0
	NC 1C - Alleles	0	0
	NC 1C- Quant	0	0
NC for duration of skin sampling	**NC 2A - Alleles**	0	0
	NC 2A - Quant	0	0.0001
	NC 2B- Alleles	0	0
	NC 2B- Quant	0	0
	NC 2C - Alleles	0	0
	NC 2C- Quant	0	0
Volunteer Thigh Swab	**Thigh Swab Alleles**	22	32
	Thigh Swab Quant	0.0019	0.0022
	Match to Patient	Match	Match

(*continued on next page*)

Table 18 (*continued*)

Location	Mock Examination Ref.	SARC L09 > 20 Airflow	SARC L06 < 20 Airflow
Air Vacuum	**Air Vacuum Control Alleles (PNC)**	Not Tested	0
	Air Vacuum Control Quant (PNC)	Not Tested	0
	Air Vacuum Sample Alleles	1	13 - Match to operator
	Air Vacuum Sample Quant	0	0.0003

Appendix 9

Table 19
Phase 2 results (as allele count and quant scores (ng/μl)) for the 5 NC samples recovered at each of the 4 SARCs.

Location	Test	Negative Controls					
Mock Examination Ref.		NC Couch Alleles	NC Couch Quant	NC Trolley Alleles	NC Trolley Quant	NC Surface Alleles	NC Surface Quant
SARC L09	1	0	0	0	0	3	0
>20 Airflow	2	0	0	1	0	0	0
	3	0	0	0	0	0	0
	4	11	0.0003	1	0	0	0
	5	8	0.0001	0	0	0	0
SARC L05	1	0	0	0	0	0	0
<20 Airflow	2	0	0	0	0	1	0
	3	1	0.0001	0	0	0	0
	4	0	0	0	0	0	0.0001
	5	0	0	0	0	0	0
SARC L06	1	0	0.0001	0	0.0001	0	0
<20 Airflow	2	0	0	0	0	1	0
	3	0	0	1	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
SARC L10	1	0	0	0	0	0	0
>20 Airflow	2	0	0.0001	0	0	0	0
	3	0	0	0	0.0003	0	0
	4	1	0	0	0	1	0
	5	0	0	0	0	0	0

Appendix 10

Table 20
Phase 2 air testing results (as allele count and quant scores (ng/μl)) for the 5 air vacuum samples and PNCs from each of the 4 SARCs.

Location	Test	Air Vacuum System			
Mock Examination Ref.		Control Alleles (PNC)	Control Quant (PNC)	Sample Alleles	Sample Quant
SARC L09	1	Not Tested	Not Tested	2	0.0001
>20 Airflow	2	Not Tested	Not Tested	0	0
	3	Not Tested	Not Tested	0	0
	4	Not Tested	Not Tested	1	0
	5	Not Tested	Not Tested	0	0
SARC L05	1	Not Tested	Not Tested	0	0
<20 Airflow	2	Not Tested	Not Tested	0	0
	3	Not Tested	Not Tested	0	0
	4	Not Tested	Not Tested	0	0
	5	Not Tested	Not Tested	0	0.0001
SARC L06	1	Not Tested	Not Tested	2	0
<20 Airflow	2	Not Tested	Not Tested	0	0
	3	Not Tested	Not Tested	1	0.0001
	4	Not Tested	Not Tested	0	0
	5	2	0	4	0
SARC L10	1	Not Tested	Not Tested	1	0
>20 Airflow	2	Not Tested	Not Tested	0	0
	3	Not Tested	Not Tested	0	0
	4	Not Tested	Not Tested	0	0
	5	Not Tested	Not Tested	1	0.0001

Appendix 11A

Table 21
The *t*-test calculations of the phase 2 air vacuum results (allele count) for the 2 SARCs with the <20 whole room replacement air flow system compared to the 2 SARCs with >20.

Alleles Count -Statistics		
Statistics	<20 Airflow	>20 Airflow
Average	0.7	0.5
Standard Deviation	1.337	0.707
Variance	1.789	0.5
Count	10	10
Coefficient of Variation	191.070	141.421
Confidence	0.95	0.05
F-Test		
F-Calculated	0	F table
***t*-Test Equal Variances**		
Spool	0.504	
t Calculated	0.2478	
t table	2.101	**The difference is not significant**
***t*-Test unequal variances**		
DF	13.666	
t calculated	0.418	
t table	2.160	**The difference is not significant**

Appendix 11B

Table 22
The *t*-test calculations of the phase 2 air vacuum results (Quant score ng/μl)) for the 2 SARCs with the <20 whole room replacement air flow system compared to the 2 SARCs with >20. Determining that the difference between is not significant.

Quant Score- Statistics		
Statistics	<20 Airflow	>20 Airflow
Average	0.00002	0.00002
Standard Deviation	4.216E-05	4.216E-05
Variance	1.778E-09	1.778E-09
Count	10	10
Coefficient of Variation	210.819	210.8189
Confidence	0.95	0.05
F-Test		
F-Calculated	0	F table
***t*-Test Equal Variances**		
Spool	1.9878E-05	
t Calculated	0	
t table	2.101	**The difference is not significant**
***t*-Test unequal variances**		
DF	18	
t calculated	0	
t table	2.101	**The difference is not significant**

Appendix 12

Table 23
Coriolis suitability air testing results from the 4 different locations, results are provided as allele count and quant score (ng/μl), the profile type of the DNA identified in these samples has been provided in the table

Sample		Quant Score	Average Quant Score	Allele Count	Average Allele Count	Profile Type
A	Bar Air Sample	0.224	–	125	–	Suitable for comparison
	Bar EM1- Bar	0.0506	0.0402	107	115	Mixed profile
	Bar EM2- Table	0.0213		105		Mixed profile
	Bar EM3- Arm of Chair	0.0487		134		Mixed profile
B	Toilet Air Sample	0.007	–	58	–	Suitable for comparison
	Toilet EM1- Door Handle	0.0105	0.0083	62	55	Low level mixed profile
	Toilet EM2- Toilet	0.0046		40		Low level mixed profile
	Toilet EM3- Tap	0.0098		63		Low level mixed profile
C	House Air Sample	0.0003	–	0	–	Not suitable for comparison
	House EM1- Window Sill	0.0054	0.0066	57	37	Low level mixed profile
	House EM2- Table	0.0004		5		Mixed profile
	House EM3- Arm of Chair	0.0141		50		Low level mixed profile
D	Garage Dust Air Sample	0.0443	–	60	–	Suitable for comparison

Appendix 13

Table 24
SARC EM criteria developed from the EM data in this study. The EM sample result criteria is determined by the allele count (above limit of detection (LOD)) and quant score (ng/μl) of the EM sample and the action required is based on the risk rating of the EM sampling location.

Category	Quant Score	Alleles	Action based on risk of location*		
			Low Risk	Medium Risk	High Risk
Green	Quant Score ≤ 0.0002	≤10 alleles above LOD	1	1	1
Amber	Quant Score > 0.0002 < 0.004	>11 <35 alleles above LOD	1	2	2
Red	Quant Score ≥ 0.004	≥35 alleles above LOD	2	3	3

*Action Level	Facility Action	FSP Action
1	No action required	No action required
2	Area forensically re-cleaned	EM sample requires profiling and checked against the SED
3	Area forensically re-cleaned and re-sampled.	EM sample requires profiling and checked against the SED

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