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ELECTROPHORESIS

RESEARCH ARTICLE

Up in the air: Presence and collection of DNA from air and air conditioner units

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Abstract

Biological material is routinely collected at crime scenes and from exhibits and is a key type of evidence during criminal investigations. Touch or trace DNA samples from surfaces and objects deemed to have been contacted are frequently collected. However, a person of interest may not leave any traces on contacted surfaces, for example, if wearing gloves. A novel means of sampling human DNA from air offers additional avenues for DNA collection. In the present study, we report on the results of a pilot study into the prevalence and persistence of human DNA in the air. The first aspect of the pilot study investigates air conditioner units that circulate air around a room, by sampling units located in four offices and four houses at different time frames post-cleaning. The second aspect investigates the ability to collect human DNA from the air in rooms, with and without people, for different periods of time and with different types of collection filters. Results of this pilot study show that human DNA can be collected on air conditioner unit surfaces and from the air, with air samples representing the more recent occupation while air conditioner units showing historic use of the room.

KEYWORDS
air sampling, eDNA, forensic science, human DNA profiling

1 | INTRODUCTION

Biological material is routinely collected from crime scenes to aid criminal investigations and judicial issues. Improvements in DNA technologies significantly expanded the kind of trace that can now provide usable DNA profiles, including touched surfaces and objects and trace quantities of DNA, where only a few cells are required to generate a profile. Currently, trace samples constitute the majority of samples processed annually; for example, 62% of all

Abbreviations: eDNA, environmental DNA.

samples processed in Forensic Science South Australia in 2020 were trace or touch evidence [1]. The terms 'touch DNA' and 'trace DNA' are sometimes used interchangeably, and 'trace' can be interpreted to mean any detectable substance or mark left by an action. In this paper, we define 'trace DNA' as a generic term to describe any type of biological material that is present in sub-optimal quantities and touch DNA as biological material that is deposited upon skin contact with a surface [2]. Thus, a touch DNA sample can also be deemed a trace sample if present in small quantities. Additionally, non-touch biological material may be present, in sufficient quantities to generate a DNA profile, on exhibits and surfaces. This biological material is neither trace nor touch and yet may constitute an important part of what is collected from crime scene surfaces. The makeup of touch deposits remains elusive but is believed to be comprised cell-free DNA, fragmented residual DNA from shed corneocytes, endogenous nucleated and anucleate cells and transferred exogenous nucleated cells [3]. Touch biological material is often present in lower quantity and quality to other sources of DNA containing biological material such as blood [2]; hence, significant interest and resources are being invested in understanding its prevalence, persistence and recovery from common forensic targets [4–7]. Touch DNA collected from crime scene surfaces is believed to originate from direct physical contact, indirect transfer via an intermediary [2], and aerosol and shedding from clothing and skin are also believed to contribute [3, 8–10].

Touch samples are frequently of poor quality (due to degradation of DNA by environmental exposures over time) and quantity (due to limited deposition) [11–16] and even if collected from the correct location frequently result in complex mixtures and partial or no DNA profiles.

To add to the complexity of obtaining useful information from samples that target touch DNA, forensic awareness amongst criminals is on the rise with criminals cleaning surfaces and wearing gloves to prevent their DNA being deposited at crime scenes [17, 18]. However, it is very unlikely that an average offender, even one with forensic awareness, could totally prevent their DNA from being released into the environment. Edwards et al. [19] found that DNA is released in tiny saliva droplets from regular breathing. In general, humans shed 2–10^8 cells a day from their epidermal layers in a process known as desquamation [20–22]. Puliatti et al. [9] found that individuals would deposit DNA to surfaces in their proximity that they had never touched. There are likely to be other mechanisms of release of cell free DNA to the environment that are not well studied, due to the difficulties in doing so. This leads to the idea that the environment itself may be able to be targeted for DNA.

Recently, Fantinato et al. [23, 24], following on non-human environmental DNA (eDNA) environmental monitoring studies [25], investigated the presence and persistence of human DNA in the air showing its potential as an investigative tool capable of detecting the recent occupants at collection locations. eDNA and eRNA are genetic material that is shed from an organism from sources such as skin, saliva and urine to their environment, including soil, ice, snow, air and aquatic [26, 27].

In the present study, presence and persistence of human DNA in the air and air conditioner units were investigated. Air collection units may represent a historical record of DNA present in the air and settled on the air conditioner surfaces during air circulation. Background samples and samples at different time points post air conditioner cleaning were collected from four houses and offices, and the quantity, quality and contributors of human DNA analysed. Further, air samples were collected using an air collection device for different periods of time, during and post participant occupation of a meeting room, using different types of filters and sample sizes to investigate collection methods targeting persons recently occupying the sampling space.

2 | MATERIALS AND METHODS

2.1 | Air conditioner units sampling from four offices and four houses

In order to ascertain the prevalence and provenance of human DNA within eDNA on air conditioner units, samples were taken from air conditioner units in four single occupation offices and four households inhabited by both adults and children (see Supporting Information 1 for sample details (1A) and air conditioning units (1B); Supporting Information 2 for demographic details). Samples (double swabbing; see Section 2.4 for details) were taken twice, first as background (T1) and again at 4-week occupation accumulation (T4). The same areas were sampled at each time point. Specifically, background DNA from the units was collected, and the units were subsequently cleaned with 1% hypochlorite, 70% ethanol and distilled water; a method known to clean surfaces of DNA [28]. Control samples were taken after cleaning to confirm the success of the cleaning, and the air conditioner units were then left to collect DNA for 4 weeks. One exception was office 3, where no control samples were taken after cleaning for the 4-week time frame; for this office control, samples taken from week 3 were tested. Office and house owners were instructed not to touch the units directly but otherwise were free to go about their normal daily activities as usual. The house spaces were used daily, and the offices were

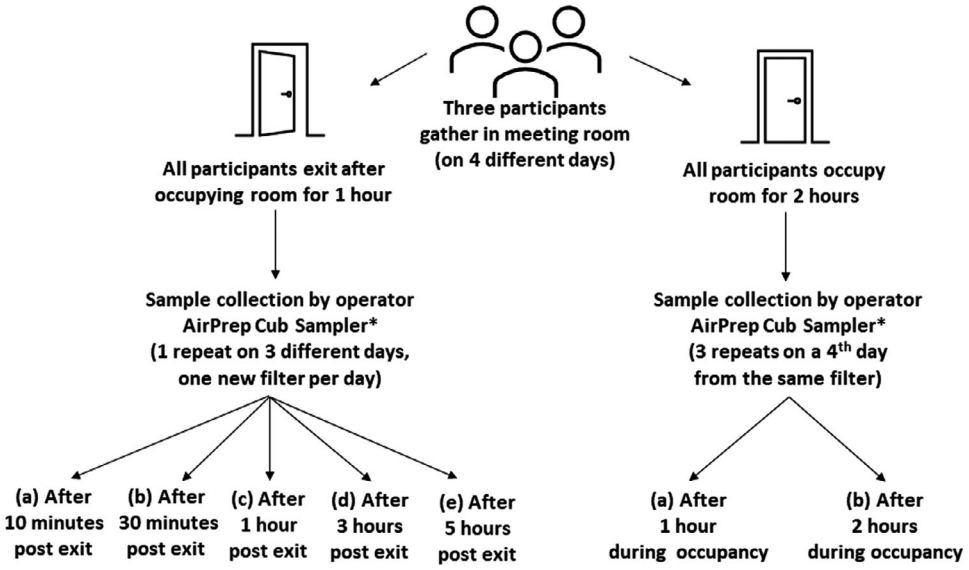

FIGURE 1 Experimental conditions details for the 4 days using one filter type and sample size. **Device was paused at each time point to collect four punch discs for DNA processing.*

generally used during working hours for 5 days per week. The precise durations and sequence of occupancy of known individuals and any visitors, or their activities, were not recorded.

2.2 | Air conditioner unit sampling from one office and one house at four different time frames

In order to ascertain the accumulation and provenance of human DNA within eDNA on air conditioner units over time, an additional set of further samples was taken from the air conditioner units in one office (office 1) and one house (house 2) at different time points post-cleaning (1 day, 1 week and 4 weeks) and included additional sampling locations on the unit at some of the time frames (see Supporting Information 3). In order to test each time frame, once the time point samples were collected, the test surface was cleaned to remove any residual DNA (as described in Section 2.1), and the air conditioner unit was left to collect DNA for the newly selected period of time. This procedure was repeated for each time point.

In total, 69 air conditioner samples were collected for human DNA detection.

2.3 | Air collection

The aim of this pilot study, in preparation for a large-scale air collection study, was to test the capability of the air collection machine, AirPrep Cub, to collect human DNA from air. In this study, different periods of occupation within a meeting room, performance of a standard DNA process and direct PCR method, DNA recovery from different size samples taken from the filter and alternative filter types were assessed. All air samples were collected using AirPrep Cub Sampler ACD 220 (InnovaPrep) with AirPrep, Millipore Durapore, Whatman Grade 1 and Whatman Nylon filters (tested due to the reduced cost of these alternative filters) that were run at maximum (200 L/min) for a specific time(s) outlined below. The researcher collecting the samples wore full PPE, including medical facemask, prior to entering the sampling location. Reference samples were collected from all participants and the researcher collecting the samples.

2.3.1 | Time study using one filter and sample size

Air samples were collected from a meeting room ($3 \times 4\ m^2$) with the device placed in the middle of the room on top of the table. All participants were sitting around the table (at a distance of ~1 m from the device) and allowed to perform office work and communicate with each other.

Twenty-one samples were collected from the meeting room, which is known to be in use by a number of different people on a daily basis, on 4 separate days.

For the first 3 days, samples were collected in five different ways (setting A; Figure 1; Supporting Information 4): Participants exit meeting room after 1 h occupation and (a) collection for 10 min after participant exit; (b) collection for 30 min after participants exit; (c) collection for 1 h

after participants exit; (d) collection for 3 h after participants exit and (e) collection for 5 h after participants exit. For each collection day, the same filter was used, and the device was stopped at each tested time point to collect the relevant sample (four punch discs [diameter: 2 mm] [Miltex biopsy tissue sampling tool; ProSicTech]) collected per sample.

A different single Airprep filter was used on day 4 with the device being paused at each collection time frame. For the fourth day, samples were collected in two different ways (setting B; Supporting Information 4A): (a) collection for 1 h while three participants were in the room working on their computers (three repeats on a single day from the same filter) and (b) collection for 2 h while three participants were in the room working on their computers (three repeats on a single day from the same filter); four punch discs (diameter: 2 mm; Miltex biopsy tissue sampling tool; ProSicTech) were punched from each filter and combined for DNA analysis. Punches were taken to extend the use of the same filter for collection at different time intervals under the same sampling and environmental conditions.

2.3.2 | Time study with different filters and sample sizes

Samples were collected from the same meeting room as Section 2.3.1 with the same device and participant placement using Airprep filter. Forty samples (30 for standard analysis and 10 for direct PCR) were collected from the meeting room in four different ways (Supporting Information 4B): (a) collection for 2 h prior to cleaning and entry of participants (background samples); (b) collection for 2 h after all surfaces were cleaned with 1% hypochlorite, water and 70% ethanol prior to participant entry; (c) collection for 2 h while 3 participants were in the room working on their computers and (d) collection for 2 h immediately after the 3 participants left the room. These samples (a)–(d) were collected on 1 day. For each collection time, the device was stopped to remove the filter and resumed immediately after the filter was replaced. A new filter was used for each test condition.

For collections (c) and (d), the experiment was repeated three more times, each with a different type of filter: Millipore Durapore filters, Whatman Grade 1 filters and Whatman Nylon filters. In collections (a) and (b), only AirPrep filters were used. Each of the substitute filter tests was run on a separate day, and all surfaces in the meeting room were cleaned with 1% hypochlorite, water, and 70% ethanol before participant entry.

After collection, for each experiment (a)–(d), filters were divided into a half, a quarter and two-eighth portions. The half, the quarter and one of the one-eighth portions were separately submitted for DNA analysis. Two punch discs (diameter: 2 mm; Miltex biopsy tissue sampling tool; ProSicTech) were punched from the remaining one eighth of filter for direct PCR analysis.

In total, 61 air samples were collected for human DNA detection.

2.4 | Sample processing and interpretation

Samples were collected with informed signed consent under the Flinders University Human Ethics Approval (4915). For air conditioner units, including their filters, samples were collected using wet and dry double swabbing technique using Forensic L/XL swabs (STARSTEDT) and sterile water for injection (2–3 drops) in individual disposable 10 mL vials (Pfizer), by traversing the whole target area multiple times with each swab, whereas each swab was regularly rotated during the swabbing action) (see Supporting Information 1). Both swabs were pooled for DNA analysis. DNA was extracted using DNA IQ System (Promega) (elution volume of 60 µL), quantified with Quantifler Trio DNA quantification kit (Thermo Fisher Scientific), amplified using PowerPlex21 kit (Promega; 30 cycles and 175 RFU detection threshold) with fragment separation and typing using G3500xl Genetic Analyser (Thermo Fisher Scientific; 24 s, 1.2 kV) and Genemapper ID-X (v1.6) (Thermo Fisher Scientific). The quantity of extracted DNA added for amplification was 0.5 ng or 15 µL if the concentration was ≤0.0033 ng/µL.

The DNA processing of the filter discs was the same as for the swabs of the air conditioner units. For the direct PCR analysis, samples were amplified without extraction or quantification using GlobalFiler kit (Thermo Fisher Scientific) in the final volume of 25 µL.

The minimum number of contributors (NOC) required to reasonably explain the DNA profiles obtained was determined, and all profiles from 1 to 5 contributors were analysed in STRmix v 2.9 (ESR and FSSA). The NOC was determined by first removing all possible artefacts and then using information from a maximum number of alleles at the locus and peak height balance information. Profiles that were deemed to originate from six contributors ($n = 6$) were re-analysed using an increased analytical threshold of 350 RFU (increased manually in STRmix setting from the standard 175 RFU) and deconvoluted as five-person mixtures with this modified baseline. Note that this practice of reanalysing samples using a higher analytical threshold is not used routinely in casework and was only done in this instance to allow the interpretation of complex mixtures for research purposes. Samples were interpreted using STRmix including mixture deconvolution, likelihood ratio

(LR) calculations and mixture-to-mixture comparisons. In places, verbal equivalents for the LR values were provided in text. The range of LR values encompassed by each verbal equivalent descriptor is provided in Supporting Information 1C. For mixture-to-mixture comparisons (LR calculations without θ), the 100 000 threshold was set for possible matches. Where a participant or participants of the study were not excluded as a contributor(s) (for LR > 1000), such samples were re-deconvoluted conditioning this donor or donors as a contributor. This was only done for the purpose of mixture-to-mixture comparison and investigation of common unknown donors. Statistical analysis (Kruskal–Wallis and Mann–Whitney; $p < 0.05$) of the results was performed using IBM SPSS.

3 | RESULTS AND DISCUSSION

3.1 | Air conditioner experiments

3.1.1 | Control samples (air conditioner units)

No DNA was detected from all but one office and all house samples that had been cleaned and for all time frames showing that the cleaning protocol was sufficient to eliminate detectable background DNA. The only exception was a control sample taken from the outer lattice after the 24-h interval in office 1 where a partial two-person mixture (0.24 ng total DNA; 21 alleles at 10/20 loci) was detected. Mixture-to-mixture comparison of this partial profile to the results generated after the unit was run for 24 h did not result in inclusionary LRs; thus, the source of this partial profile remains unknown. As the remaining controls are taken after 1 and 4 weeks from the same location all produced negative results, this residual DNA was removed during subsequent office cleaning.

3.1.2 | Air conditioner units sampling from four offices and four houses

Detectable amounts of DNA were found in all offices and houses in all background and 4-week samples, and this resulted in interpretable profiles from all but one sample (Table 1). The NOC in these samples ranged from 0 to 5 people. Seventy-seven per cent of office samples (10/13) where profiles were generated and 9% of house samples (1/11) were partial profiles which was also reflected by the quantification results where, on average, approximately 74% more DNA was detected in the house setting (see Supporting Information 7 for two examples of profiles generated). Slight-to-moderate degradation was noted in both offices and houses (Table 1) indicating that biological material may persist at the targeted air conditioner locations in relatively good condition with minimal degradation. Air conditioning units are usually positioned inside the house, often away from direct sunlight, and these conditions are known to be favourable for DNA persistence.

These results show that DNA can collect on the air conditioner unit, and the likely source of this DNA is aerosolised biological material. Specifically, the air conditioner units were not directly contacted (experimental instructions) and, in general, were located either high on the wall or on the ceiling and thus out of reach of the participants. Thus, the only means for the DNA to collect on the sampled surfaces was for the DNA shed via aerosol, clothing and skin to move with the draft and settle on the sampled area.

On average, there was more DNA detected in the background samples than 4 weeks after cleaning, but these differences were not statistically significant (Figure 2). The median amount of DNA was 0.82 ng (0.12–8.88 ng) and 0.48 ng (0.06–1.62 ng) for offices and 9.42 ng (1.14–18.24 ng) and 1.5 ng (0.96–4.38 ng) for houses at T1 and T2. Overall, significantly more DNA ($p = 0.004$) was detected in the houses than offices, and re-accumulation was quicker in the houses, as evident from the total amounts of DNA, where at 4 weeks offices had 18% and houses 23% of the total amounts detected before cleaning. It is possible that the differences seen are a result of larger number of people residing in houses and/or different types of activities performed in these different locations. Office spaces are usually associated with computer work, among others, whereas houses are associated with more diverse pastimes such as exercise, food preparation and intimate activities. However, this study investigated a small number of each type of premises, and further studies are needed to confirm these differences.

There did not appear to be any consistency in the owner's DNA accumulation over the 4-week test period (Table 1). In a number of the test locations, initial background testing either resulted in exclusion or LRs of low strength compared to those observed at 4 weeks, as seen in house 1 (outer) and house 2 (inner; both owners). In contrast, at a number of locations, background samples resulted in a high level of detection with much lower or exclusionary LRs after cleaning, as can be seen in office 2 (inner L) and house 3 (inner). These results show that although sufficient DNA can be collected, in forensically relevant quantities, over just a few weeks, the amount and the likelihood of such deposition are likely to be affected by the frequency and duration of occupation and the actions of the persons whose DNA is being collected. Furthermore, environmental conditions such as a presence of draught or items that have DNA of interest in the vicinity of the collection point may affect the amount and

TABLE 1 DNA results obtained from the four tested offices and houses including total amount of DNA, degradation index (DI) and number of contributors detected during background (T1) and 4-week sampling (T2) and interpretation data of mixture-to-mixture comparison over 100 000 threshold (common unknown contributors) and the owner(s) likelihood ratio (LR) results at T1 and T2.

Location/time	Background (T1)				4 weeks (T2)				Interpretation
Sample location	*Amount (ng)*	*DI*	*Cont.(n)*	*Owner LR's T1*	*Amount (ng)*	*DI*	*Cont.(n)*	*Owner LR's T2*	*Mx to Mx (LR > 100 000); unknown sources (u/k)*
Office 1 Inner L	8.88	9	5	LR = 6E−04	0.24	1.5	1	LR = 0	Nil
Office 2[a] *Inner L*	3.06	4.9	5	LR = 4E08	0.06	0	0	n/a	Nil
Outer L	0.732	2.1	4	LR = 1E12	1.14	3	3	LR = 1E06	
Inner B	0.3	1.9	2	LR = 3	n/a	n/a	n/a	n/a	
Outer B	0.12	2.4	1	LR = 4	n/a	n/a	n/a	n/a	
Office 3 Inner L	0.12	1.8	2	LR = 130	0.66	5.3	2	LR = 2E13	Nil
Outer L	0.9	1.8	3	LR = 13E15	0.3	5.1	2	LR = 5E11	
Office 4 Inner L	8.76	1.8	2	LR = 1E25	1.62	1.9	3	LR = 3E22	T1 and T2 Inner LR = 2E08 *(C2-u/k)*
House 1[b] *Outer*	1.14	1.7	3	O1LR = 0 O2LR = 0	0.72	1.1	3	O1LR = 40 O2LR = 50	T1 and T2 Outer LR = 3E13 *(C1-u/k)* T1 and T2 Outer LR = 3E08 *(C2-u/k)*
House 2[b] *Inner L*	18.24	1.3	3	O1LR = 10	4.38	1.3	3	O1LR = 2E06	Nil
Outer L	15.54	1.2	3	O2LR = 9.5E−06 O1LR = 10 O2LR = 2E−11	n/a	n/a	n/a	O2LR = 5E11	
House 3 Inner	10.9	1.2	3	LR = 1E09	1.5	1.8	3	LR = 4	T1 Outer and T2 Inner LR = 3E28 *(C2-u/k)*
Outer	7.98	1.1	3	LR = 1E14	n/a	n/a	n/a		T1 Inner and T2 Inner LR = 2E28 *(C2-u/k)*
Filter	9.42	1	4	LR = 1E14	n/a	n/a	n/a		T1 Filter and T2 Inner LR = 1E14 *(C2-u/k)*
House 4 Inner	2.64	1.5	4	O1LR = 1E03 O2LR = 8E02	0.96	1.8	3	O1LR = 4E09 O2LR = 6E02	Nil

[a]Some offices had extra samples taken at T1; in these instances, samples were coded as L = lattice; B = blade; n/a – no sample taken.

[b]O1, O2 = owner one, owner two for houses with multiple people providing DNA reference.

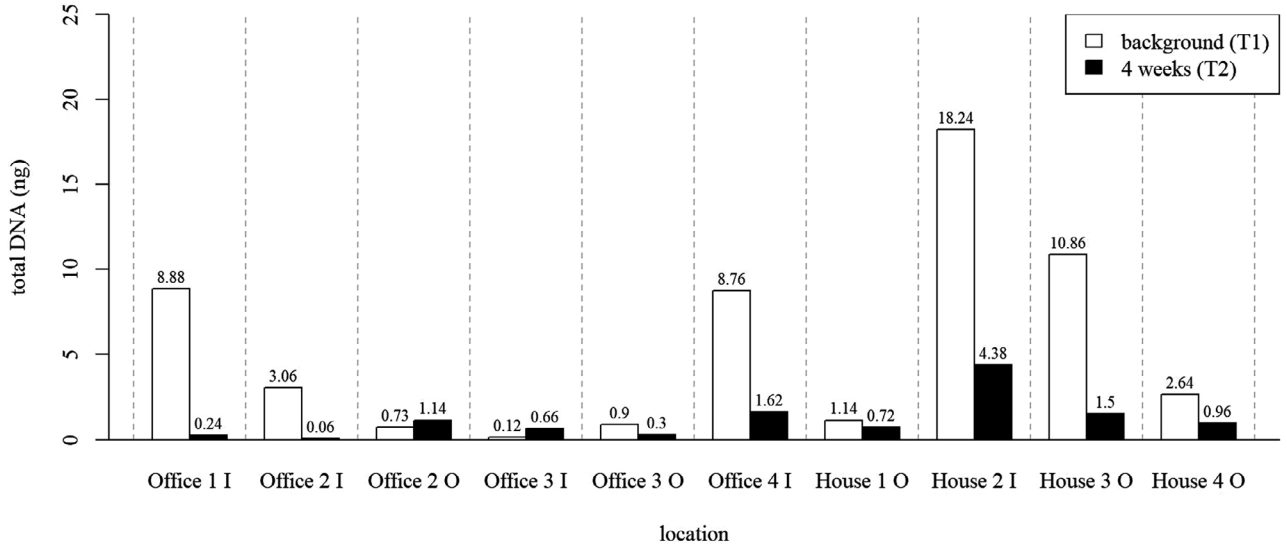

FIGURE 2 Total amount of DNA detected in offices and houses before (background) (T1) and 4 weeks after cleaning (T2) (O = outer surface sample; I = inner surface sample).

type of biological material deposited on the air conditioner units [10].

Comparing the four offices that were all of a similar size and had identical air conditioner units placed in a similar location (above the door), the length of occupation appears to play the most significant role in the identity of people whose DNA was collected. Offices 2–4 were all known to be occupied by the same owners for many years (see Supporting Information 1). The owners were detected as the main contributor in background samples and in most instances sufficient DNA accumulated to identify these individuals after 4 weeks of occupation. In contrast, office 1 was occupied by the current owner for only 1 year, and this owner (a known poor to intermediate shedder) was not detected at either time frame. Notably, there was a prominent male profile (further discussed in Section 3.2) that was detected in most background samples, but not at any other time point after cleaning. It is possible that this male profile is from the previous owner of the office who had a much longer duration of occupation and perhaps was a better shedder. Interestingly, in office 4, a non-owner unknown contributor was detected in background and 4-week samples (Mx to Mx), suggesting that one does not have to be the owner of the space to be detected. A visitor directly transferring their DNA or indirect transfer from, for example, an office owner's partner, to the space can result in detection giving credence to the author's suggestion that air DNA collection can be used for covert and intelligence purposes to ascertain recent visits to a location [23, 29, 30]. However, it is unclear why common unknown contributors were not detected in the other three offices that also had frequent visitors during the sampling time. It is possible that the individual detected in office 4 visited more frequently during the tested 4 weeks and/or was a higher shedder compared to others. Further studies are required to understand what factors influence recent visitor detection.

All four houses had children present at the premises ranging in age from 1 to 17 years. Although no DNA reference samples were collected from underage individuals, based on the DNA profiles available from the adults, there was a strong indication that children were detected in most house samples either as contributor 1 or 2 (indicated as unknown contributors in Table 1), except for house 3, where the unknown contributor appeared to be unrelated to the male house owner (who was the only DNA reference available for that house). This unknown contributor from an apparent female may have been the female owner of the house who did not provide a reference sample; however, this could not be confirmed in the absence of her sample. The apparent detection of children in most samples is in line with studies that show that children tend to deposit more DNA and are, in general, higher shedders than adults [31, 32].

3.1.3 | Air conditioner unit sampling from one office and one house at four time frames

Detectable amounts of DNA (>0 ng) were found in most samples from office 1, and this resulted in positive typing results in 88% of the samples (Table 2; Figure 3A; Supporting Information 5A for Mx to Mx results). The NOC in these samples ranged from 0 to 5 people. Eighty-eight per cent of these samples either produced no or partial profiles.

TABLE 2 DNA results obtained from the different locations on the air conditioner unit of office 1 taken before (T1; background) and at three different time points after cleaning (T2–T4) including total amounts (ng) and degradation index (DI; in brackets), number of contributors (cont.), mixture-to-mixture comparisons (same location at four time frames) and likelihood ratio (LRs) to the owner.

Sample type/time	Background (T1)		1 day (T2)		1 week (T3)		4 weeks (T4)		Interpretation	
	ng (DI)	cont.	ng (DI)	cont.	ng (DI)	cont.	ng (DI)	cont.	Mx to Mx	Owner LRs
Lattice inner left	8.88 (9)	5	0.42 (5.6)	2	0.24 (1.4)	3	0.24 (1.5)	1	Nil	Exc. all
Lattice inner right	3.72 (4.1)	5	0.24 (3.8)	1	0 (3.8)	0	0.24 (7.5)	2	Nil	T1LR = 30 T2, T3 exc. T4LR = 3
Lattice outer left	1.26 (2.9)	4	0.06 (9.4)	1	0.12 (3.1)	2	0.06 (2.9)	2	Nil	T1, T2, T3 exc. T4LR = 5
Lattice outer right	7.98 (13.5)	4	0.12 (9.9)	2	0.06 (8.2)	0	0.3 (6.2)	2	Nil	T1, T2, T3 exc. T4LR = 2E04
Blade inner	0.66 (4.6)	3	0.06 (0)	0	0.24 (1.9)	2	0.18 (3.1)	1	Nil	T1LR = 4E03 T2, T3, T4 exc.
Blade outer	0.06 (3.4)	1	0 (0)	0	0.06 (15.9)	0	0 (0)	0	Nil	Exc. all
Filter left	15.36 (3.2)	5	n/a	n/a	n/a	n/a	n/a	n/a	Nil	Exc.
Filter right	11.52 (2.7)	5	n/a	n/a	n/a	n/a	n/a	n/a	Nil	Exc.

Note: n/a – no sample taken; for simplicity, LRs favouring exclusion were reported as exclusion.

Slight-to-moderate degradation was detected in most samples from office 1 with two samples indicating severe degradation (outer right lattice at T1 and outer blade at T3; Table 2). This office was unoccupied and unused for approximately 1 year prior to the latest owner moving in, and it is possible that some of the collected samples are older than the samples collected from other locations.

There were significantly different amounts of DNA and NOC detected among the four time frames (excluding filters that were sampled only at T1). Background samples had significantly more DNA and higher NOC than either 1 day ($p = 0.02$ and $p = 0.02$; amount of DNA and NOC respectively), 1 week ($p = 0.02$ and $p = 0.02$; amount of DNA and NOC, respectively) or 4 weeks ($p = 0.03$ and $p = 0.02$; amount of DNA and NOC, respectively) which were not significantly different from each other. The median amounts of DNA recovered and NOC for the four time frames were 2.5 ng (0.06–8.88 ng)/4 (1–5) contributors (T1), 0.09 ng (0–0.42 ng)/1 (0–2) contributor (T2), 0.06 ng (0–0.24 ng)/1 (0–3) contributor (T3) and 0.21 ng (0–0.3 ng)/1 (0–2) contributor (T4) (Figure 3A).

Mixture-to-mixture comparisons of samples from office 1 taken from different surfaces and different time frames did not result in any matches above the designated threshold, apart from the background samples. Thus, it appears that different individuals were detected at different times of the experiment. This office is frequently visited by various students, staff and cleaning personnel (usually one at a time for brief periods), and these people were possibly detected at different times and locations representing the different histories of the office use at each collection time; however, reference samples from these aforementioned people were not available to test this hypothesis. It should be noted that most of the profiles generated at T2 to T4 were partial in nature and may not have had sufficient information at corresponding loci to generate inclusionary LRs. In contrast, Mx to Mx comparison of background samples to each other provided inclusionary LRs (above threshold) from all but one (outer blade) of the samples (Supporting Information 5A; Figure 4A).

Contributor one (highest Mx) in the remaining seven background samples appeared to be from the same male source producing inclusionary LRs ranging from 1E05 to 5E12 (average 5E11). It is possible that this male donor was the previous long-term resident of the office. This suggestion is supported by the fact that this contributor was not detected in any of the samples once the background DNA was removed. Further, in two of the background filter samples, two common additional donors (perhaps persons associated with the previous owner) were detected (contributor 2 in both samples with LR 2E06; and contributors 4 and 5 with LR of 1E06).

Detectable amounts of DNA were found in most samples from house 2, and this resulted in positive typing results in all but one sample (Table 3; Figure 3B). The NOC in these samples ranged from 0 to 4 people. Apart from one negative result, no partial profiles were detected for house

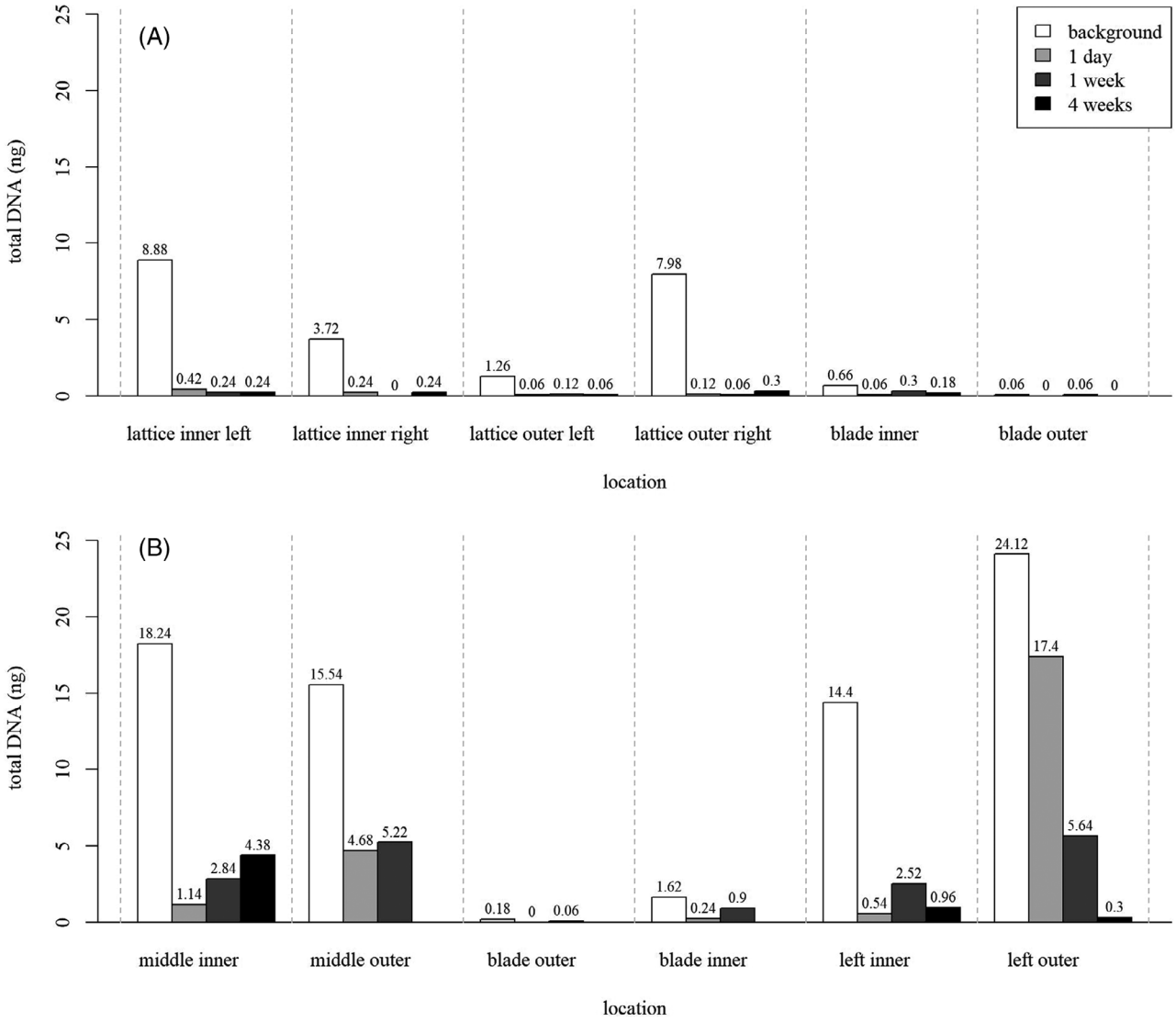

FIGURE 3 Total amount of DNA detected in (A) office 1 at six locations (inner left and right lattice (1 and 2); outer left and right lattice (3 and 4) and inner and outer blades (5 and 6)), (B) house 2 at six locations middle (inner and outer); blade (inner and outer) and left side (inner and outer); at four time frames.

samples. Very mild degradation was noted in the samples taken from the house (Table 3).

There were no significant differences in amounts of DNA and NOC detected among the four time frames taken from house 2. The average amounts of DNA and NOC for the four time frames were 14.97 ng (0.18–24.12 ng)/3 (2–4) contributors (T1), 0.84 ng (0–17.4 ng)/2.5 (0–4) contributor (T2), 2.4 ng (0.06–5.64 ng)/3 (1–3) contributor (T3) and 0.96 ng (0.3–4.38 ng)/2 (2–3) contributor (T4).

Data showed that, in the office (Table 3), and for all test surfaces combined, less than 10% of initial biological material had re-accumulated after cleaning for most samples (ranging on average from 4% to 5% of the amounts detected in background samples). For the house, greater DNA amounts accumulated post-cleaning (ranging on average from 15% to 32% of the amounts detected in the background samples). Similar amounts of DNA at the three different times post-cleaning suggested that, at least for these two locations, 4 weeks did not provide sufficient time for the amounts of DNA to return to the pre-cleaning levels.

Mixture-to-mixture comparisons of the samples from house 2 taken from different surfaces and different time frames resulted in multiple matches (Supporting Information 5B; Figure 4B). This space has been occupied by the same owners for over 10 years and includes two children as part of the household. Reference samples from children were not available for comparison, and it is possible that they have contributed to the DNA detected on the air conditioner unit.

Results from these two locations (office 1 and house 2) showed that sufficient DNA, to generate a DNA profile, can accumulate over time; however, the length of time

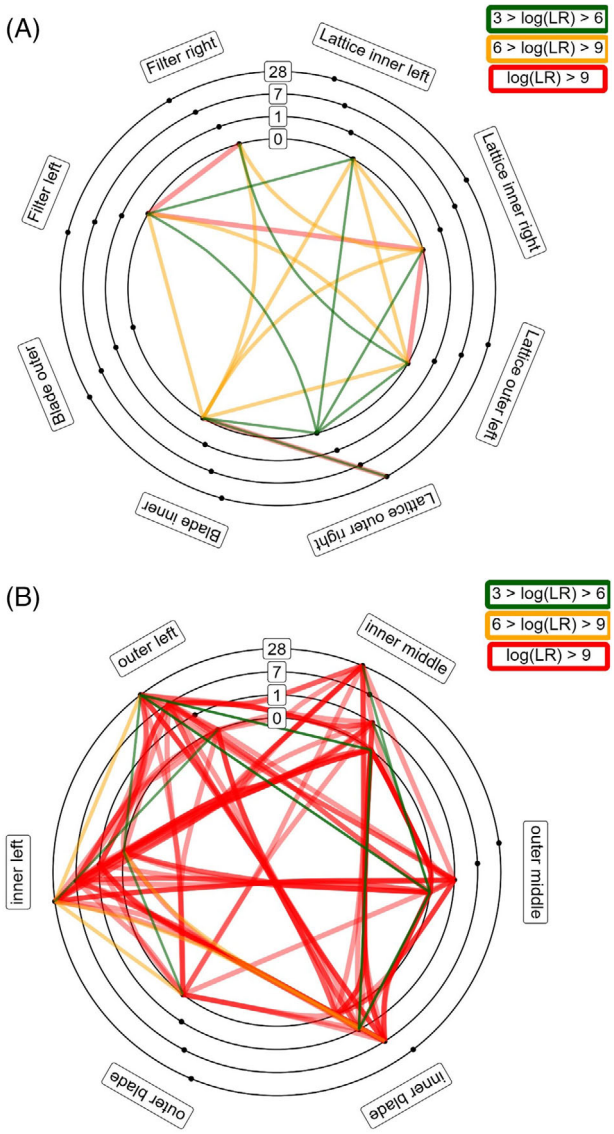

FIGURE 4 Ringed plot showing results of mixture-to-mixture comparison of all samples taken from (A) office 1 and (B) house 2. The rings separate the sampling times (with days listed in the labels at the top of the rings, where 0 = background). The points on the rings are placed under headings for each sampling location. The colours of the links between time and location are coloured according to strength as given in the legend.

required to detect an individual is likely dependent on the use of the space, the type and use of air conditioner unit and the persons involved. Owner of office 1 was either excluded or provided LRs with moderate-to-very strong support (one sample at T4) for their presence; notably, these non-exclusions were background or 4-week samples; suggesting that prolonged time may be required for re-accumulation. This is in contrast to the results from house 2 and some of the other offices and houses where owners were detected at most times tested post-cleaning. House 2 owners were detected at each time frame post-cleaning including day 1, the shortest time frame with many results providing extremely strong support for H1. There are a number of possible reasons for this discrepancy. First, the occupation of houses and other offices was much longer than the 1 year reported for office 1. Longer occupation can result in the accumulation of person's DNA on items and surfaces, and this DNA may be dislodged and re-located, for example, to the air conditioner unit arising from person's movements and draughts from open windows and doors and use of the air conditioner itself. Second, the shedder status of the participants in this study was not specifically tested; however, it was known (from their participation in prior, unpublished shedder studies) that office 1 owner was a poor to intermediate shedder, and this could have exacerbated the lack of detectable DNA in this office. However, it is not known if an individual's environmental and touch shedder status display similar behaviour. Furthermore, two of the three owners in the other offices and all houses had male owners, and these individuals are possibly higher shedders compared to their female counterparts [31, 33–35].

3.1.4 | Overall observations from air conditioning units

Overall, lattice samples had more DNA than on the blade, possibly due to the smaller sample area of the blades surface (Tables 1–3). Furthermore, the blade is the only surface in the air conditioner unit that moves when the unit is activated, and this can dislodge DNA that was initially deposited, resulting in loss and redistribution of the initially deposited biological material. The inner surfaces, in general, had more DNA than outer surfaces, perhaps from being protected from the actions such as drafts that can dislodge DNA. However, the comparison of inner and outer surfaces in the house and office spaces was a lot more variable. Filters had the highest amount of DNA, which is expected as fibres of the filter can trap cells and DNA retaining it for later sampling and making this a favourable surface type for future air sample collection methods. It would be useful, in any further studies, to test the collection of DNA on different air conditioner filters over time, something that was not done in the present study.

3.2 | Air samples

3.2.1 | Time study using one filter and one filter sample size

No DNA was detected in the three samples of the filter taken 10 min after the three participants left the meeting

TABLE 3 DNA results obtained from the different locations on the air conditioner unit of house 2 taken before (background) and at three different time points after cleaning (T2–T4) including total amounts (ng), number of contributors (cont.) and likelihood ratio (LRs) to two adult owners (O1 and O2).

Sample type/time	Background (T1)		1 day (T2)		1 week (T3)		4 weeks (T4)		Interpretation
	ng (DI)	cont.	ng (DI)	cont.	ng (DI)	cont.	ng (DI)	cont.	Owner LRs
Inner middle	18.24 (1.3)	3	1.14 (2)	3	2.28 (1.1)	3	4.38 (1.3)	3	T1LR P1 = 10; P2 = exc T2LR P1 = 1E04; P2 = 4E03 T3LR P1 = exc; P2 = exc T4LR P1 = 2E06; P2 = 5E11
Outer middle	15.54 (1.2)	3	4.68 (2.1)	4	5.22 (1.3)	3	n/a	n/a	T1LR P1 = 10; P2 = exc T2LR P1 = exc; P2 = 4E05 T3LR P1 = exc; P2 = exc T4 n/a
Blade inner	1.62 (1.3)	3	0.24 (2.4)	2	0.9 (1.3)	3	n/a	n/a	T1LR P1 = 1E04; P2 = 4E05 T2LR P1 = 2E04; P2 = exc T3LR P1 = 60; P2 = 2E04 T4 n/a
Blade outer	0.18 (2.3)	2	0 (0)	0	0.06 (1.1)	1	n/a	n/a	T1LR P1 = 30; P2 = E2E03 T2LR P1 = n/a; P2 = n/a T3LR P1 = exc; P2 = exc T4 n/a
Inner left	14.4 (1.4)	3	0.54 (2)	2	2.52 (1.4)	3	0.96 (2.1)	2	T1LR P1 = 4E4; P2 = exc T2LR P1 = 10; P2 = 5E07 T3LR P1 = 1E06; P2 = 3E04 T4LR P1 = 1E03; P2 = 1E10
Outer left	24.12 (1.3)	4	17.4 (1.8)	3	5.64 (1.3)	3	0.3 (1.9)	2	T1LR P1 = 1E09; P2 = 1E08 T2LR P1 = 5E05; P2 = 7E03 T3LR P1 = 1E03; P2 = 6E07 T4LR P1 = 50; P2 = 6E05

Note: n/a, no sample taken or no profile generated for interpretation; for simplicity, LRs favouring exclusion were reported as exclusion (exc).

room and one of the three samples taken with participants in the room for 1 and 5 h after participant departure (Table 4). Detectable DNA was recovered in the remaining meeting room samples ranging from 0.06 to 0.36 ng total DNA, and DNA profiles generated had 0 to 4 contributors (Table 4); most of these samples were very partial in nature. Mx to Mx comparisons were not performed due to limited profiling information for most of these samples (range of detected alleles 2–39; av. of 13). None or mild degradation was detected in these samples.

Notably, all participants were excluded from these samples with the exception of three samples (LR 5–14) (Table 4). The investigator was also not excluded in three samples (LR 14–2E03); likely from contamination during set up (Table 4).

Overall, longer air collection periods appear to increase the amounts of DNA detected both with and without participants in the room; however, it is likely that amounts of DNA recovered were affected by the very small sample size as larger filter size samples recovered larger quantities of DNA (see Section 3.5.2).

3.2.2 | Time study with different filters and sample size

Detectable amounts of DNA were recovered from the Airprep filter with increasing size of the sample taken from the filter resulting in greater DNA amounts and generally higher NOC (Table 5). Light-to-moderate degradation was detected in these samples. Highest DNA amounts were collected, whereas the participants were in the room which also resulted in the detection of one of the three participants with extremely strong support. Moderate inclusionary LRs of participants were obtained in some of the other samples including background and immediately after participant exit.

No DNA was detected in samples from two of the alternative filters with very limited profiles observed with the third, Whatman Grade 1, filter indicating that these filters are not a suitable replacement for the Airprep filter.

Mixture-to-mixture comparisons identified common unknown contributor(s) between

TABLE 4 DNA results obtained AirPrep filter samples (four punches) at two different durations of room occupation (1 and 2 h) and at multiple times post 1 h occupation (from 10 min to 5 h) including total amounts (ng) and degradation index (DI; in brackets), number of contributors (cont.) and likelihood ratio (LRs) to the three participants (P1 to P3) and the researcher (P4).

Sample	Total DNA (ng) (DI)			Number of contributors			LR inclusions		
Replicate number	1	2	3	1	2	3	1	2	3
Air collected for 10 min after participants exited the room	0 (1.5)	0 (2.9)	0 (3.2)	0	0	0	n/a	n/a	n/a
Air collected for 30 min after participants exited the room	0.06 (5.6)	0.06 (1.8)	0.06 (1.5)	1	0	1	P4 = 14 All others excluded	n/a	All excluded
Air collected for 1 h after participants exited the room	0.06 (1.5)	0.06 (0.7)	0.12 (1.8)	0	2	1	n/a	All excluded	All excluded
Air collected for 3 h after participants exited the room	0.18 (3.2)	0.18 (1.8)	0.12 (1.1)	1	2	1	All excluded	All excluded	All excluded
Air collected for 5 h after participants exited the room	0.9 (1.3)	0.24 (1.3)	0.12 (0.9)	0	4	4	All excluded	P2 = 7 All others excluded	P4 = 26 All others excluded
Air collected for 1 h with all participants in the room	0.06 (2.1)	0.06 (1.4)	0 (1.4)	1	2	0	All excluded	P2 = 5 All others excluded	n/a
Air collected for 2 h with all participants in the room	0.12 (0.9)	0.36 (1.3)	0.12 (1.6)	3	3	2	P2 = 12 All others excluded	All excluded	P4 = 2E03 All others excluded

- Background and post clean samples (1/2 and 1/2 Airprep filters; LR 2E05)
- Post-clean samples (1/2 and 1/4 Airprep filters; LR 1E05 and 1/2 and 1/8 Airprep samples; LRs 3E05 and 4E05)
- Post-clean and in the room samples (1/2 and 1/2 Airprep filters; LR 6E05)
- Participants in the room samples (Airprep filters 1/2, 1/4 and 1/8; LRs ranging from 1E5 to 1E25).

Direct PCR results concurred with the results of the standard DNA analysis where all three substitute filters failed to produce informative results (Supporting Information 6). Of interest, the participant that was detected with extremely strong support with conventional analysis was not detected in the corresponding direct PCR samples. It is possible that this is the result of the small sample size of the filter that was processed during direct PCR analysis; missing the parts of the filter that collected DNA of that participant. Mixture-to-mixture comparisons of direct PCR samples identified a common unknown contributor in background Airprep sample and in the room Whatman Grade 1 filter sample (LR 3E05).

These results show that DNA can be collected from the air; however, optimization may be required to improve the quality of the generated results. The preliminary data shows that the size of the sample may play a role as the participant detected with the larger size samples (half to one eighth of the filter) was not detected where only two punches were taken for direct PCR analysis. However, it appears that eighth sized samples were sufficient and comparable to larger samples. The relevance that sample size has on the quality of the DNA profiles was also confirmed in a recent study [23] where whole Airprep filters were processed (1 filter = 1 sample) and overall provided interpretable DNA profiles and inclusionary LR results. However, Airprep filters are quite absorbent, and thus, larger sample sizes will require increased amounts of consumables to saturate the sample. Thus, the optimization of the sampling that utilises the smallest size sample that will still generate an informative profile will be of relevance, prior to large-scale implementation of this sampling technique. Additionally, different types of filters from those tested here warrant further investigation.

In these experiments, DNA from participants was not detected during DNA analysis (with high statistical support), apart from the Airprep filter samples taken, whereas participants were in the room for 2 h. However, this was a limited pilot study, and further investigations are required to better understand different variables, such as the history of space occupation (e.g. personal spaces and offices rather than meeting rooms), presence of personal items, participant shedder status and activities performed which may affect what is detected and when.

TABLE 5 DNA results obtained from four different filters sampled at different times pre, during and post participants' occupation including background, post-cleaning, 2 h with participants in the room and for 2 h immediately after participant exit including total amounts (ng) and degradation index (DI; in brackets), number of contributors (cont.) and likelihood ratios (LRs) to the three participants (P4 to P6) and the researcher (P7).

Conditions	Total DNA (ng) (DI)			Number of Contributors			LR Inclusions		
Size of filter per sample	**1/2**	**1/4**	**1/8**	**1/2**	**1/4**	**1/8**	**1/2**	**1/4**	**1/8**
Background sample with Airprep filter collected for 2 h before participant arrival	0.9 (1.6)	0.48 (1.2)	0.3 (2)	5	4	2	All excluded	P4 = 2 All others excluded	All excluded
Post cleaning sample with Airprep filter collected for 2 h after cleaning and before participant arrival	1.02 (1.2)	0.78 (1.5)	0.36 (1.7)	5	5	3	All excluded	All excluded	All excluded
Sample collected with Airprep filter with 3 participants in the room for 2 h	4.14 (1.5)	2.22 (1.4)	2.22 (1.4)	5	6	6	P5 = 6E12 All others excluded	P5 = 4E12 P7 = 4 All others excluded	P5 = 3E15 All others excluded
Sample collected with Airprep filter for 2 h immediately after participant exit after 2 h occupation	1.62 (1.2)	0.96 (1.3)	0.48 (1.5)	6	6	4	P5 = 4 P7 = 7 All others excluded	P5 = 12 P4 = 6 All others excluded	P7 = 5 All others excluded
Sample collected with Millipore filter with 3 participants in the room for 2 h	0 (0)	0 (0)	0 (0)	0	0	0	All excluded	All excluded	All excluded
Sample collected with Millipore filter immediately after participant exit after 2 h occupation	0 (5.6)	0 (0)	0 (0)	0	0	0	All excluded	All excluded	All excluded
Sample collected with Whatman Grade 1 filter with 3 participants in the room for 2 h	0.12 (1.6)	0.06 (7.5)	0 (3)	2	1	0	P6 = 6 All others excluded	All excluded	All excluded
Sample collected with Whatman Grade 1 filter for 2 h immediately after participant exit after 2 h occupation	0.12 (1.6)	0.06 (1.2)	0 (0.3)	1	1	0	All excluded	All excluded	All excluded
Sample collected with Whatman Nylon filter with 3 participants in the room for 2 h	0 (0)	0 (0)	0 (0)	0	0	0	All excluded	All excluded	All excluded
Sample collected with Whatman Nylon filter for 2 h immediately after participant exit after 2 h occupation	0 (0)	0 (0)	0 (0)	0	0	0	All excluded	All excluded	All excluded

This study shows that DNA can be easily collected from air in different occupation spaces with some optimisation. Further recommendations to optimise collection strategies will require further investigations.

4 | CONCLUDING REMARKS

This study showed that human DNA can be collected from air and on surfaces that move air, such as air conditioner units, and can identify the usual users of the space as well as frequent visitors. DNA accumulated within a fairly short period of time with owners being identified after only 4 weeks of use of the tested space. Sampling of air conditioner units may be used to identify usual or long-term occupiers of a space, and air sampling may assist in the identification of short-term or recent users of a room. Such sampling may be considered, for example, if it is suspected, gloves were worn or that the crime scene has been cleaned post incident. Further studies are required to deduce the optimal positioning of the air collection device within various types of space of potential interest and the appropriate duration of collection within various settings and circumstances to acquire the DNA of interest if present.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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