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DNA contamination of mortuary instruments and work surfaces: a significant problem in forensic practice?

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Abstract A study of 20 mortuaries was undertaken to assess whether contamination of instruments, tables and cutting areas due to residual material containing human DNA after routine cleaning is an actual or only a theoretical problem. Of the 20 mortuaries studied, 50% were found to have material containing quantifiable human DNA on the instruments and surfaces sampled. This DNA was amplified and found, in some cases, to have been derived from at least three people. Of those that did not yield measurable amounts of DNA, a number of samples were selected at random, amplified and were found to produce partial profiles indicating the presence of low levels of human DNA. The possible sources of human DNA from mortuaries are discussed as well as means to reduce or irradicate the problem of instrument contamination. Finally the implications of these findings for forensic investigations are discussed.

Key words DNA · Human Mortuary Instruments

Introduction

Since the potential forensic applications of DNA fingerprinting were first highlighted by Gill et al. in 1985 [1], the analytical techniques used to identify human DNA have developed sequentially to modern day short tandem repeats (STRs) [2, 3]. During this evolution the techniques have become quicker and more sensitive with the sample size becoming smaller. These techniques have been embraced by forensic investigators world-wide and are used nowadays to assist in the exclusion and identification of criminal offenders. Today the search for offender human DNA at a crime scene or autopsy is routine, with scientists potentially able to detect both nuclear and mitochondrial DNA from a wide range of cellular sources.

However, as techniques become ever more sensitive, the potential problem of contamination may become an issue. Recently in two areas of the United Kingdom human DNA profiles from samples submitted during autopsy procedures were found to have arisen from human DNA contaminating dirty mortuary instruments. Since these incidents the Home Office has instructed all pathologists that disposable instruments should be used where possible when taking samples which could be submitted for DNA analysis.

A study was undertaken to establish whether these two episodes were unrepresentative, reflecting isolated mortuary practices, or whether there is widespread contamination of mortuary instruments and work surfaces with human DNA. The discussion considers ways of eliminating potential contamination and the significance to the investigation of identifying rogue DNA from a mortuary sample.

Materials and methods

A total of 20 mortuaries used in suspicious death investigations by 5 regional Police forces were visited by a Home Office pathologist. These visits occurred as part of a suspicious death investigation necessitating a Home Office autopsy or as an unannounced visit. As the morticians had no prior knowledge of the intention of the project, the surfaces and instruments at the mortuaries visited were assumed to be in a state representative of normal practice.

On arrival the project was explained to the duty mortician and informed verbal consent to sample the instruments and surfaces gained. The instruments sampled were selected by the mortician with the basic instructions being to identify those instruments to be used during the forthcoming autopsy although all instruments offered for use had to have been used and cleaned at least once during their previous working life in the mortuary. Brand new instruments were not accepted and a search of the mortuary to specifically identify dirty instruments was not performed.

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At each mortuary the same type of instruments were sampled. These consisted of a pair of coronary artery scissors commonly used to sample fingernails, a large pair of scissors, a large organ/brain knife and a pair of toothed forceps. In addition to these the mortuary table and cutting area were sampled.

Sampling was performed using cotton swabs moistened with tap water. A control tap-water swab was taken at each mortuary. During the sampling process the pathologist wore a new pair of sterile latex gloves on each occasion. The whole cutting surface of the instrument was swabbed and in the case of scissors they were opened fully so that the hinge areas could be exposed and sampled. Random areas of the mortuary table on which the autopsy was to be performed and the cutting area were sampled. All swabs were then frozen at –20 C prior to analysis. A blood sample and buccal (mouth) swab from the pathologist were submitted as controls.

The samples were extracted in four batches using the indirect chelex method [4]. Samples were quantified using a primate-specific alpha satellite probe assay [5]. The DNA extracts were amplified using the Second Generation Multiplex (SGM) system [6]. All samples which contained a measurable amount of DNA were amplified, as well as a random batch of samples where a quantification value could not be assigned i.e. DNA may still have been present but at less than 100 pg/μl. The PCR products were run on Applied Biosystems automated DNA sequencers, model ABI prism 377 [7].

Finally the methods routinely used to clean and sterilise the mortuary instruments as well as the method for cleaning and sterilisation used by the analytical laboratory were recorded.

Results

A total of 20 mortuaries were visited over a 2-month period. Of these, 17 mortuaries had all 6 swabs taken. Of the mortuaries, three had only five swabs taken; one had no large knife swab, one had no cutting area swab and one had no coronary artery scissors swab taken (Table 1). This yielded 117 test swabs and 20 control swabs.

Of the 20 mortuaries analysed, 10 (50%) had quantifiable human DNA on one or more of the instruments, cutting area or mortuary table sampled. The measurable DNA ranged from 0.25–2.5 ng/μl (Table 1). All of the control swabs from tap-water were negative.

Only one mortuary (No. 5, Table 1) had measurable DNA on the coronary artery scissors. This particular mortuary also had the largest number of contaminated instruments or surfaces of all mortuaries visited and was the only site to yield positive samples from toothed forceps and a cutting area.

Only two mortuaries had measurable DNA on a large knife or mortuary table (Nos. 3 and 17, Table 1). The most common instrument to be contaminated with human DNA in the survey was large scissors with eight mortuaries yielding positive results.

Of the 13 swabs (11%) that gave positive results all were amplified and all produced full human DNA profiles. In each case the profile was from a different person, thus no two profiles were the same, four (31%) gave profiles consistent with 1 person, five (38%) gave a mix of at least two people and four (31%) gave a mix of at least three people (Table 2). Analysis of the pathologists' control samples showed that none of the profiles identified had arisen from contamination by the pathologist during the sampling process.

Table 1 DNA quantification results from swabs taken at all 20 mortuaries (*CAS* coronary artery scissors, *LS* large scissors, *TF* toothed forceps, *LK* large knife, *MT* mortuary table, *CA* cutting area, *C* control swab; *0* <100 pg/μl)

Mortuary	Quantification results (ng/μl)						
	CAS	LS	TF	LK	MT	CA	C
1	0	0	0	0	0	0	0
2	0	1.5	0	0	0	0	0
3	0	0	0	0	1.0	0	0
4	n/a	0	0	0	0	0	0
5	1.0	2.5	2.5	0	0	0.5	0
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0	1.0	0	0	0	0	0
9	0	0	0	0	0	0	0
10	0	0	0	0	0	n/a	0
11	0	0	0	0	0	0	0
12	0	0.5	0	0	0	0	0
13	0	0.25	0	0	0	0	0
14	0	0	0	n/a	0	0	0
15	0	1.0	0	0	0	0	0
16	0	0	0	0	0	0	0
17	0	0.5	0	0	0	0	0
18	0	0	0	2.5	0	0	0
19	0	0	0	0	0	0	0
20	0	0.5	0	0	0	0	0

Table 2 The number of profiles identified from each source which yielded a positive DNA quantification result

Mortuary	Sample	Profile
18	Large knife	1 Person
8	Large scissors	1 Person
13	Large scissors	1 Person
17	Large scissors	1 Person
5	Large scissors	Mix of at least 2 people
5	Toothed forceps	Major/minor mix of at least 2 people
5	Coronary artery scissors	Major/minor mix of at least 2 people
2	Large scissors	Major/minor mix of at least 2 people
15	Large scissors	Low level mixture of at least 2 people
5	Cutting area	Mix of at least 3 people
12	Large scissors	Mix of at least 3 people
3	Mortuary table	Mix of at least 3 people
20	Large scissors	Mix of at least 3 people

Of the 104 swabs where the quantification results had no value assigned, 24 random swabs were amplified of which 8 (33%) gave a low level partial profile indicating that although no quantification results were obtained there was some low level human DNA (<100 pg/μl) present in these samples.

All of the mortuaries had a protocol for cleaning and sterilising their instruments (Table 3) and 18 used a disinfectant (usually 1% phenol) with or without the use of a detergent and/or autoclaving. The two remaining mortuar-

Table 3 Routine procedures for cleaning and sterilising instruments in each mortuary and the analytical laboratory

Mortuary	Method used routinely for cleaning and sterilising instruments
1	Wash in detergent then washed in disinfectant. PM40 handles autoclaved
2	Wash in detergent then washed in disinfectant
3	Wash in disinfectant only
4	Wash in disinfectant and then autoclave
5	Wash in disinfectant only. Autoclaved used for infective cases
6	Wash in disinfectant only
7	Wash in disinfectant only
8	Wash in disinfectant only
9	Wash in disinfectant only. Autoclaved used for infective cases
10	Wash in disinfectant and then autoclave
11	Wash in detergent then washed in disinfectant. Autoclave used for infective cases
12	Wash in disinfectant and then autoclave
13	Wash in disinfectant only. Autoclaved used for infective cases
14	Wash in disinfectant only. Autoclaved used for infective cases
15	Wash in detergent and then autoclave
16	Wash in detergent then washed in disinfectant
17	Wash in detergent and then autoclave
18	Wash in disinfectant only
19	Wash in disinfectant and then autoclave
20	Wash in disinfectant and then autoclave
Analytical laboratory	Remove any gross contamination with Microsol 3. Soak in Microsol 3 for 30 min. Wash well in sterile distilled water, wrap in foil and bake at 120 °C for a minimum of 12 h

ies used a detergent only in combination with autoclaving, eight mortuaries used an autoclave on every occasion with an additional five using one in known infective cases for example hepatitis viruses or Creutzfeldt-Jakob disease. In four cases (Nos. 12, 15, 17 and 20) an autoclave was routinely used yet quantifiable DNA was found to be present on large scissors.

Discussion

Workers within the field of DNA research have recognised since 1985 the need for strict laboratory practices related to DNA extraction and amplification because of the potential problem of contamination of the procedures both by human and non-human DNA from the worker and laboratory environment [8]. As the techniques used during criminal investigations have become so sensitive, the problem of human DNA contamination has become an issue of critical importance in the event that a potential offender DNA profile is considered to have been identified from a sample derived from a scene of crime or mortuary.

Once a human DNA profile has been identified from a crime scene or autopsy sample the source of the DNA must be explored. This is both an expensive and time-consuming exercise. The first source to be considered and excluded is that it has arisen during the sampling or analytical process. Workers within the forensic field could have their DNA profiles on a database for exclusion purposes although this would remain the choice of the individual. Although most investigators will have their conventional fingerprints on a database, to date the placement of their DNA on such a database is the exception. Thus we would recommend that it could be considered that all such investigators could submit DNA samples to a database for exclusion purposes.

Toledano et al. [9] reported the potential for the accumulation of human DNA on the structural facilities of mortuary rooms and raised the possibility of DNA contamination from these sources. However, to date the possibility that the DNA profile could have arisen from the instruments used to obtain the sample in the mortuary has not been addressed in the literature. Although individual cases have come to notice in the United Kingdom in recent months, a survey such as ours has not to our knowledge been performed as most people have not previously considered this obvious source of potential contamination.

If a profile is found from a sample taken from a mortuary then there are six potential sources. This does not include the possibility that the DNA has arisen from contamination of the corpse by contact with the inside of an unclean body bag, as it is the practice of some mortuaries to reuse body bags which may not have been sterilised properly between bodies. Thus in criminal investigations the head, hands and feet must always be placed inside new, sterile bags and then the body be placed into a new body bag or plastic sheeting.

The first potential source from within the mortuary environment is that the DNA has arisen from the deceased's own cells which have contaminated the sampling process. This may particularly occur with fingernail samples. This source of the DNA, however, is easily identifiable by comparing the profile with that generated from the control blood sample taken at the mortuary during the autopsy. This sample, however, must be taken with a new sterile disposable syringe and be placed into a new sterile container which in turn must be appropriately labelled with the details of the deceased and site from where the sample was taken.

If the profile did not originate from the deceased then there are five other potential sources. The first is that it has arisen from the pathologist during the sampling procedure. This source again can be excluded if the pathologist's own DNA profile is known. The mortician is the second source to be considered. Whilst everyone is concentrating on potential sources of contamination at the time of the autopsy one must not forget that the mortician may have handled the instruments already, without gloves on during the cleaning process or whilst putting out the instruments prior to the autopsy. As (to date) the mortician's profile is also unlikely to be known, this may produce a profile which may be incorrectly considered to have arisen from a possible offender. Thus morticians could

also be considered to have their DNA profiles placed on an exclusion database.

The most interesting possibility is that the DNA relates to a third party i.e a potential assailant. Unless the third party has handled the instruments used to sample for DNA and thus potentially left DNA on the instrument, this profile could be used to try to identify a third party and be used in any subsequent criminal proceedings.

The next source is from the structural elements of the mortuary as highlighted by Toledano et al. [9] with the final source of DNA from the instruments themselves. The problem that this will cause to the investigators is, as with the above two possibilities, that this will identify a profile from an unknown person(s). This could have three potential consequences. The first is that it could result in a costly and time-consuming search of the National DNA database or even result in regional population screening to try and identify a third party that is already buried or cremated. Thus, unless by chance, the assailant has the same profile as that of a source within the mortuary this will prove an expensive and fruitless activity. The second to be considered is that as, to date, human DNA profiles are not unique then theoretically the offender may have the same profile as that of the source on the instrument. Thus, although this is a remote possibility, a trial defence that the profile was derived from sample contamination rather than from the assailant could theoretically be offered. Finally, in cases were the body is decomposed and solid tissue, for example psoas muscle, may be required to assist with identification, if the deceased DNA was present in insufficient quantities then the contaminant DNA may be in the majority and thus lead to potential problems related to the identification. It is thus important that any such sample should be recovered using sterile pincettes and disposable, sterile scalpels and placed in an appropriately labelled sterile container. Thus instrument contamination is of importance and may not only affect the cost and time taken to investigate the crime but eventually contribute to a failed investigation.

We have shown that at least 50% of mortuaries had quantifiable human DNA on instruments and mortuary surfaces. Thus this study confirms that this is a real problem and does not reflect isolated mortuary practice. This fact may unfortunately come as no surprise to many workers within forensic pathology.

Only one mortuary of those sampled had human DNA on the coronary artery scissors, the most frequently used instrument to take samples to search for third party DNA in mortuaries. It should be noted that this mortuary had the largest number of contaminated instruments of the whole survey. The other instrument frequently used to sample fingernails are large scissors. This was the most frequently contaminated instrument and we would thus recommend that these are not used for such a purpose unless they have been cleaned properly before use. The other instruments and surfaces sampled are not usually used during sampling for third party DNA and thus these results indicate the extent of the problem but may not have a bearing on an investigation.

The amplification results are of great interest. They show that not only are the instruments contaminated but that, in some cases, three or more separate profiles were present. This reflects the standard of mortuary instrument cleaning and sterilisation after previous autopsies.

Two important issues have arisen from this study. The first is to draw attention to investigating personnel of the potential problem of human DNA contamination of mortuary instruments and work surfaces. The second is to consider ways to eradicate the risk. One would think that the only way to completely eradicate the problem is to use brand new disposable instruments for each new autopsy for any procedure where a DNA sample is retrieved. However, although this instruction has been issued by the Home Office for England and Wales, the question then arises as to whose responsibility it is to provide the instruments and who is to bear the cost; the mortuary, the pathologist, the scientists or the police. Even if one is to use brand new instruments some of the manufacturers of disposable instruments will not guarantee the user that they are free of DNA. If this is true then there may be no point in using any such instruments unless a guarantee that they are DNA-free can be assured.

Another problem that may arise is that surfaces with which the body or samples may come into contact may be contaminated and if several cases are being done at one mortuary then the team may potentially run out of clean instruments.

A different solution is to recognise the potential problem and assume that all instruments and surfaces are contaminated by human DNA. If new instruments are then unavailable, the instruments available to be used can be swabbed prior to the procedure. This will provide a control swab to check any DNA profile against in the laboratory and thus if a profile is identified the instrument can be eliminated or confirmed as the source. The action of swabbing may also assist in cleaning the instruments prior to use.

The next solution is to prevent contamination by improving decontamination/sterilisation procedures related to mortuary instruments and the cleaning of work surfaces. However, to date, no specific work has been published to assess the most efficient and reliable way of cleaning and sterilising mortuary instruments. Our project does not assist us in this task although it does appear that the use of disinfectants for example 1% phenol or hypochloric acid with or without detergents, may reduce the risk although it does not guarantee that the instruments will be DNA-free. It does, however, highlight the fact that autoclaving does not necessarily remove the risk of instrument contamination by human DNA. The more extreme practice of the analytical laboratory may prove reliable but in practice may not be achievable by most mortuaries [10].

The final solution is to centralise autopsy services to one regional mortuary as occurs in many countries. This will allow this mortuary to maintain the highest standards of cleanliness and sterilisation of instruments and work surfaces and be stocked will all equipment necessary to

maintain a modern criminal autopsy investigation. Specific sets of instruments can be identified and maintained for the sole purpose of specimen sampling where DNA may be an issue and a stock of appropriate disposable instruments maintained. Regular checks could then be made to ensure that DNA contamination of instruments and surfaces within the mortuary whenever possible did not occur.

This study has highlighted potential problems and solutions related to human DNA contamination of instruments and surfaces in modern mortuaries. Although it does reflect on the quality of cleanliness in mortuaries, the sensitivity of the techniques are such that the source of the DNA may not be apparent to the human eye and thus work areas and instruments may appear 'clean'. A practical approach by investigators should be considered to minimise or eradicate this potential source of rogue DNA. The findings and implications of this study should be considered on a global scale by all who are involved by criminal investigation. It does not only affect those dealing with deceased persons but also clinical practice as highlighted by studies addressing false-positive PCR results from 'sterile' bronchoscopes [11, 12]. Thus it must be considered that sampling for example, fingernails, in the living may also be subject to contamination unless new instruments are used.

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