

1 DNA contamination – a pragmatic clinical view

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Contamination considerations

Forensic DNA testing has had a significant impact on the investigation of crimes. Initially developed in the mid-1980's by Alec Jeffreys and his group (Jeffreys, Wilson and Thein, 1985), the original 'fingerprinting' procedure has been modified and refined to the point where 'DNA profiling' has seemingly attained infallibility status (Koehler, 1993; National Research Council, 1996; Thompson, 1997). This status has been acquired and/or enhanced by its use in the exoneration of persons falsely convicted of various crimes and the subsequent successful conviction of the apparent perpetrators (Innocence Project, 1992). The concept of DNA infallibility in the investigation of crimes has, however, led to some significant miscarriages of justice. It is these exceptions that serve as a warning that the use of DNA profiling in criminal investigations requires a full understanding of the process by those employing the technology, and caution in the interpretation of the findings and their subsequent application in the legal system.

Different types of error may occur in any scientific procedure; DNA testing is no exception. DNA profiling is not and never has been infallible. There are many potential sources of error in its application. If it is to be applied competently within the legal system, all persons utilizing DNA data must have some understanding of the science behind it and of its potential sources of error. What is apparent is that many involved in the process, whether for specimen collection, testing, interpretation, application or presentation in court, have a poor understanding of DNA technology and its limitations, the potential for both error and misinterpretation, and the consequences of these when judged in a court of law. The sources of error are many and it is not our intention to examine them all. Instead, this chapter will address the issue of DNA contamination of items – other than their deliberate contamination – and consider this in relation, in particular, to the work of the forensic physician.

Does DNA contamination occur? The answer is yes, and there are some very high-profile cases to demonstrate its occurrence – probably it occurs far more frequently than we appreciate. The following examples of accidental contamination illustrate

how easy it is for contamination to occur and how DNA profiling results may too readily be accepted without question.

The 'Phantom of Heilbronn'

Between 2007 and 2009, the German police were confronted by an apparent unknown female criminal who had been linked by DNA to some 40 crime scenes over a fifteen-year period (BBC News, 2009; Himmelreich, 2009). These crimes, which included six homicides and various burglaries and break-ins, had occurred in locations across Austria, France and Germany. Dubbed the 'Phantom of Heilbronn' following the murder of a young policewoman, Michele Kiesewetter, in Heilbronn in 2007, the hunt for this mystery female intensified and a substantial reward of €300,000 was offered for information leading to her capture. In 2009, the German police eventually unearthed the identity of the murderer and thief. The answer had been in plain sight all the time and is a stark reminder of the fallibility of DNA profiling.

In March 2009, while investigating the case of an unidentified, burned, deceased male from 2002, the Phantom's DNA profile was detected when re-examining the fingerprints of a male asylum-seeker taken years earlier in order to reveal his DNA profile. The improbability of this finding resulted in the DNA test being repeated using a second swab to collect more DNA from the specimen. This time the Phantom's DNA was not identified. The problem was revealed as one of DNA contamination and the identified DNA was that of a female worker on a production line for cotton-tipped swabs – swabs used by various police forces across Europe to collect specimens at crime scenes.

Stefan König of the Berlin Association of Lawyers was reported (Himmelreich, 2009) to have made a very pertinent comment in relation to placing too much credence on identifying trace amounts of DNA:

What we need to avoid is the assumption that the producer of the traces is automatically the culprit. Judges tend to be so blinded by the shiny, seemingly perfect evidence of DNA traces that they sometimes ignore the whole picture. DNA evidence on a crime scene says nothing about how it got there. There is good reason for not permitting convictions on the basis of DNA circumstantial evidence alone.

His comment has proved to be correct and the question of how the DNA 'got there' is particularly pertinent.

The Jama case

On 21 July 2008 a young Somali male, Farah Abdulkadir Jama, was found guilty and imprisoned for the rape of a 48-year-old female in a Melbourne nightclub on 15 July 2006. The sentence was for six years with a non-parole period of four years. It was alleged that Mr Jama had sexually assaulted the woman, who had been found unconscious in a toilet cubicle, locked from the inside. Although the woman had

consumed alcohol that evening, its effects were in excess of her expectations and raised the possibility that she had been drugged, presumably by her drink or drinks being spiked. Subsequent toxicological analysis did not support this assumption but did show a high blood alcohol level.

The normal procedure for cases of acute alleged sexual assault in the State of Victoria is that victims are taken to one of several designated examination suites located within some of the major metropolitan hospitals. Within these suites a forensic examination is undertaken by a forensic medical officer (FMO) or forensic physician (FP) and specimens are collected and provided to the police. This occurred in the case of this female and Mr Jama's DNA was identified during subsequent forensic laboratory testing of biological specimens from the alleged victim. As a result, Mr Jama was found guilty of a sexual assault and sentenced to imprisonment, of which he completed approximately fifteen months prior to his being acquitted by the Court of Appeal.

The case against Mr Jama had been based on a single piece of evidence: that his DNA had been identified on a single slide preparation and associated swab collected, among other swabs, from the alleged victim when examined by the FMO. The matching DNA was, in fact, recovered from only one of two swabs taken from the endo-cervical region of the woman and was not present on two other swabs taken from within the vagina. In addition, from the available information, his DNA did not appear to be on the alleged victim's clothing. Also of importance was that Mr Jama had never attended the nightclub in question (based on evidence tendered to the court) and there was no evidence to suggest otherwise.

Following Mr Jama's acquittal, the Hon. Justice Vincent undertook an enquiry on behalf of the Victorian Department of Justice (Vincent, 2010). He came to the opinion that the most likely cause for Mr Jama's DNA appearing on the swab was as a result of contamination within the clinical examination suite. Part of the reasoning supporting this was a failure to find any evidence to suggest that Mr Jama's DNA could have come in contact with material from the alleged victim after the swabs had been packaged in any other way, and, more significantly, that the FMO undertaking the examination of the alleged assailant had undertaken a similar examination of another woman about one day previously in the same examination suite. This female had engaged in a sexual activity with Mr Jama, an issue that had not led to any charges being placed.

It is of note that Mr Justice Vincent was of the view that:

Not only was the evidence against him [Mr Jama] ultimately revealed as inherently unreliable but, it came to be recognized, the likelihood that he had committed the offence allegedly was, at the risk of understatement, remote and found to have been perpetrated in circumstances where it seemed to be highly improbable that there was any crime at all.

Mr Justice Vincent went on to say:

In other words, the DNA evidence was, like Ozymandias' broken statue in the poem by Shelley, found isolated in a vast desert. And like the inscription on the statue's pedestal, everything around it belied the truth of its assertion. The statue, of course, would be seen by any reasonably perceptive observer, and viewed in its surroundings, as a shattered

monument to an arrogance that now mocked itself. By contrast, the DNA evidence appears to have been viewed as possessing an almost mystical infallibility that enabled its surroundings to be disregarded. ... After following this history of the proceedings against the unfortunate Mr Jama from their origins through to their disastrous conclusion of his conviction, I have been left with the deep impression that at virtually every point, and by almost everyone involved, it was handled with so little insight into the issues which it presented that no need was seen to explore further or conduct research into them. This was particularly so in the case of those involved in the legal processes. There were ample warning signs along the way that suggested that something was amiss but they were simply not read.

These views are very similar to Stefan König's in relation to the Phantom case (discussed above) and have echoes in the case that follows. In contrast, it was the disbelief of a police officer in the Adam Scott case (discussed below) that revealed another case of contamination.

Mr Justice Vincent made a number of recommendations for change in relation to the examination facilities, specimen collection equipment and the legal processes. Importantly, as the swabs and slides used for the collection of DNA evidence were taken from readily and generally accessed trolleys located within the examination suites rather than from dedicated DNA collection kits, recommendations made were that the specimen collection equipment be kept separate from the trolleys and in locked storage accessed only by the examining doctor. Further, it was recommended that any unused specimen collection equipment prepared for an examination be disposed of.

Following tabling of Mr Justice Vincent's report, the Victorian Department of Justice implemented many of the recommendations (Smith, 2011) as well as the development and implementation of a forensic medical examination kit. This is now in use in examination suites throughout Victoria.

LGG forensics

In October 2011, Mr Adam Scott was charged with the rape of a female in Manchester, UK. He was arrested and held in custody by the Greater Manchester Police based on DNA evidence alone, which had been obtained from a vaginal swab taken from the alleged victim. Mr Scott, who lived in Exeter, denied ever having been in Manchester and an analysis of his mobile telephone by the police indicated that he was not in the area of the reported rape. The detective leading the investigation voiced concern regarding the reliability of the DNA result and the matter was subsequently investigated (Rennison, 2012).

The case was reviewed and, after further DNA testing, the probable cause of what was in fact contamination leading to the incorrect charging of Mr Scott was that within the testing laboratory there had been an inappropriate reuse of a disposable plastic tray, which had then contaminated the sample from the female. Mr Scott's DNA was in the system within the laboratory as he had been involved in an incident involving British Transport Police, during which there was spitting, resulting in his DNA sample being submitted for assessment. Mr Scott's saliva DNA sample, with its high-level male DNA, from this earlier, unconnected incident had been present in one of the wells in the automated tray that was incorrectly reused in the laboratory, the

same one that the sample from the alleged female victim (actually containing only her DNA) was subsequently loaded. The error was revealed when the reports of this fully accredited laboratory were reviewed. Initially, retesting of the original DNA extract confirmed the original findings of Mr Scott's potential culpability. This extract, however, had already been contaminated due to the reuse of the plastic disposable tray. When this possibility was raised, a complete retesting of the original swabs from the female was undertaken; these showed that Mr Scott's DNA was not present at all. The charges against him were subsequently withdrawn.

Jaidyn Leskie

Jaidyn Leskie was a 14-month-old child. On 15 June 1997, he disappeared while in the care of a family friend. Five and a half months later, Jaidyn's body was found floating in Blue Rock Dam, Victoria, in the district in which he had lived and was being cared for. Various investigations were made into the child's death and the findings were reported by the Victorian State Coroner in 2006 (Johnstone, 2006). According to the report, a search of the dam where Jaidyn was found revealed the presence of a plastic bag containing some of his clothing. On the bib and tracksuit a female DNA profile was identified and, in subsequent investigations in the early 2000's, a search of the Victoria Police Forensic Services Centre's (VPFSC) database revealed a match to the DNA profile of a female rape victim. Police investigation indicated that this woman was unlikely to have had links to the toddler. This raised the possibility of contamination. The matter was addressed in the Coronial investigation and involved the testimony of experts on the subject from a variety of laboratories. The VPFSC argued that contamination was unlikely given the processes that they engaged in and considered that the profile from the bib and track-suit was not the identified female but that of another, unidentified individual, the result of what they referred to as an 'adventitious match'.

The DNA aspect of the case was interesting as the VPFSC continued to maintain the adventitious match hypothesis rather than admit to a possible contamination within the laboratory, despite the fact that clothing from both cases – the child and the rape victim – was received within seven minutes of each other, and that the two cases were examined by the same person within a relatively short time-frame, albeit in different locations within the laboratory. It was possible that a single pair of scissors, wiped clean between each use with 70% alcohol (which, it should be noted, does not denature DNA), may have been used to cut cloth samples from each case. In light of the evidence, it is difficult to accept the adventitious match hypothesis.

The four cases outlined above illustrate how contamination can occur in different settings. The first is where contamination takes place in the preparation of materials being used for the purpose of DNA collection; the second is of the potential for cross-contamination in forensic examination suites; and the third and fourth show how contamination can occur within the laboratory setting. The last does occur occasionally and processes are implemented within laboratories in an effort to detect such contaminations. Irrespective of where the contamination occurs, however, it will

never be detected if those investigating it do not consider the possibility seriously and remain open to the potential for contamination to take place in their own domain.

DNA profiling

The first use of DNA in criminal justice was when Alec Jeffreys used his DNA ‘fingerprinting’ technique to assist in the identification and conviction of Colin Pitchfork for the rape and murder of two young women in Leicestershire in 1983 and 1986. The technique involved the identification of a particular DNA sequence through the use of a radioactive-labelled complementary sequence, or multi-locus probe, within variable lengths of DNA cut with restriction enzymes (known as restriction fragment length polymorphisms). Electrophoresis pulls the charged DNA through a gel matrix, with the fragments being spread out according to size. Fragments containing the bound probes can be visualized by exposure to an x-ray plate and are seen as dark bands, which can be likened to a bar code. The resulting pattern is known as a DNA fingerprint and is identical within an individual and essentially unique. This provides a powerful tool if the DNA fingerprint from a crime scene matches that of a suspect. However, despite its accuracy and reproducibility, the technique requires DNA in microgram amounts and is slow.

Two scientific developments have since changed DNA analysis into the DNA profiling technique used today:

- The polymerase chain reaction (PCR) has enabled picogram amounts of DNA to be multiplied, incorporating fluorescent probes into the copies, thus allowing visualization.
- The discovery of polymorphic mini-satellites within the genome. Mini-satellites are repeated sequences of 2–5 nucleotides (the building blocks of DNA) and are known as short tandem repeats (STRs). Probes are prepared that bind to the sequence outside the repetitive region and the PCR reaction makes copies of the repeat sequence. The STR and its flanking sequence are selected so that they are found only once in the genome. The different-sized molecules produced reflect the number of repeats and these are separated by means of electrophoresis. A DNA profile is a list of numbers of repeats, each obtained from a selected area of the genome.

Sample collection

Perhaps the most important part of the process in DNA profiling is at the beginning of the process. The integrity and preservation of collected DNA, representing what is believed to be material likely to be associated with the crime scene, is vital. With the ability to profile DNA from just a few cells, individuals responsible for law enforcement and crime scene management need to be acutely aware of the ease with which DNA on an item can be transferred to other areas, or contaminated with extraneous and unrelated DNA, as illustrated in the Jama case discussed above.

In addition to protective clothing and defined processes to avoid contamination, discussed in more detail below, the integrity and documentation of material collected directly from the crime scene requires:

- A detailed record of what was found, with annotated photographs, sketches and measurements.
- Items of evidence collected into clearly and appropriately labelled individual paper containers which are securely sealed to maintain a proper chain of custody. Plastic bags are to be avoided as any retained moisture can speed DNA degradation.
- Use of a double swabbing (wet then dry) technique to collect biological stains from items that cannot be moved. DNA-free water opened at the scene is used to rehydrate biological material followed by a dry swab to collect any remaining cells. The choice of swab material is important in the subsequent analysis, as is the need for the swab to be left to dry. DNA survives better when dry, avoiding hydrolytic cleavage of the sugar-base bond which leads to loss of the base and subsequent single strand breaks in the DNA molecule. (This is discussed in more detail below.) Drying also protects the material from any DNA-digesting enzymes that may be prevalent from contaminating bacterial growth.

DNA extraction and quantification

DNA extraction methods are designed to separate proteins and other cell components from the DNA molecule and to remove any inhibitors of the PCR process. All samples must be handled carefully to avoid contamination, regardless of what extraction method is used. (Laboratory protocols are considered below.)

Quantification is important as non-human DNA may be extracted, along with human material. It is important in the subsequent analysis to know how much human DNA is present. Methods are also available to inform the scientist about how much male DNA is present and to provide information about the presence of PCR inhibitors.

DNA amplification

This process not only multiplies the DNA present for analysis, but also selects the particular DNA sequence to be analysed and incorporates fluorescent chemicals in the subsequent amplicon to allow downstream analysis. Essentially, some 30 heating and cooling cycles in a reaction with the appropriate chemical constituents and a polymerase enzyme (required to assemble the DNA chain) doubles the selected sequence, producing in excess of several million copies of the targeted sequence. Direct amplification methods have been developed which can decrease the risk of contamination by avoiding the extraction processes described above.

PCR offers both advantages and disadvantages:

- Very small amounts of DNA can be analysed, down – potentially – to single cells.
 - This highlights the risk of the presence of contaminating cells which are then analysed along with the intended DNA, leading to significant problems in interpretation and underlines the importance of consumables and reagents being DNA-free (Gill, Rowlands, Bastisch et al., 2010). As the ‘Phantom’ case above illustrates, gross contamination can also result if manufacturers do not treat their products to remove any DNA. This happened in the United States where a DNA-concentrating device used in several laboratories was grossly contaminated with DNA from a female member of their staff (Butler, 2012).
 - Negative controls are used to support the absence of reagent contamination.
 - Particularly important in low template analysis, where amplification is increased or DNA detection is enhanced, is a repeat analysis which can help control for environmental or plastic consumable contamination.
- Fragmented DNA molecules can be analysed, down to a few hundred base-pairs.
- Different DNA sequences can be multiplied at the same time (multiplex PCR).
- Primers are selected that target only human-specific sequences.

However, amplification may fail if:

- The PCR process is inhibited due to the DNA molecule being damaged by exogenous factors. This is also the aim of cleaning processes.

The very high template amplicons that result following PCR also provide a high risk for contamination. It is for this reason that laboratories have a strict policy of separation of areas, equipment and protective clothing, and use one-way movement through the laboratory between pre- and post-amplification facilities.

DNA separation and detection

Electrophoresis is the process used to separate different-sized DNA molecules, such as those DNA template copies in the amplicon. The separation process has to be sufficient to allow the resolution of DNA template copies that differ by only a single base in their sequence length. This is achieved using very fine long capillaries.

DNA is introduced to the capillary through an electro-kinetic injection and the electric charge draws the material through. At a particular point along the capillary the fluorescent label (fluorophore), introduced into the template during amplification, is detected by laser excitation and a photosensitive detector. Smaller molecules arrive at the detector first and the time of detection is used to assign size (length) of

the detected molecule by adding an internal calibrating size standard to each sample. These standards contain DNA fragments labelled with a different fluorescent molecule of known length. Most DNA molecules analysed in DNA profiling are between about 100 and 500 base-pairs in length.

The DNA profile

A DNA profile consists of a series of numbers, each representing one of a pair of short, repeated sequence template copies in an amplicon produced in a single multiplex PCR reaction. The particular STRs are chosen because they can be easily amplified due to their small repeat size, providing molecules of around 100–500 bp) and, with its flanking sequences surrounding the repeats, provide a unique sequence within the genome to be amplified. Smaller-sized STRs mean that even highly degraded DNA can be analysed.

A number of different STRs are chosen for the profile, with the requirement that they are inherited independently and differ sufficiently in size between individuals (i.e., are polymorphic). Generally, that will mean choosing loci (defined sequences to be amplified) that are from different chromosomes or are far apart on the same chromosome.

Tetra-nucleotides, made of four nucleotides repeated one after the other in tandem, are the most popular for human identification because of their ability to multiplex and also their reduced level of amplification artefacts. The number of repeat units determines the name of the allele. The number of repeats is highly variable and this makes them very useful for human identification. Each STR will provide a pair of alleles (represented by a pair of numbers), one from each paired chromosome, inherited from each parent, so that half of the alleles are represented within the father and half within the mother.

The DNA profile set depends on the particular STRs chosen to multiplex. Internationally, standardization is beginning to emerge. Sixteen loci, plus a sex marker, containing twelve ESS (European Standard Set) loci is widely used across Europe. In the United States there are thirteen CODIS (Combined DNA Index System) loci, eight of which are in common with those used in Europe. Manufacturers are now producing multiplex kits that contain 23 loci to incorporate both CODIS and ESS loci, providing further international standardization. Several loci still in use probably would not be included today if a new multiplex was developed, but it is because they have been used in many of the DNA databases worldwide that they need to be retained, such is the power of detection in countries that use databases. A 23 locus STR will, therefore, provide a DNA profile with 46 numbers. The chance of any one person sharing the same full set of numbers is very unlikely as a 23-locus set profile is virtually unique (less than about 1 in 10^{25}) based on an unrelated Caucasian population).

DNA interpretation

A considerable amount of expert interpretation of the profile is often needed before any analysis of DNA can be reported, especially if the sample is mixed or of low quality. The analyst must learn to differentiate artefacts from low-level DNA

components, developed through laboratory validations, before any statistical assessment can be made. The possibility of contaminating DNA components adds to the difficulty in interpretation and the risk is increased when analysing low-level DNA, even where the best anti-contamination measures have been implemented.

Until recently, it has not been possible to evaluate statistically many complex profiles containing low-level DNA; this often presents in mixtures of more than two people. Probabilistic analyses are now being employed using both semi-continuous and continuous mathematical models, ensuring that the courts can be presented not only with the evidence, but also with a fair assessment of the importance of the evidential profile.

DNA deposits

Locard's principle states that perpetrators of a crime will leave some trace at the crime scene and take some trace from the crime scene with them. This process, although initially applying to macroscopic evidence, is pertinent in relation to the subject of DNA. (For the purposes of this discussion, the terms alleged offender and offender and alleged victim and victim will be referred to as offender and victim.)

The human body is made principally of nucleated cells which will, under normal circumstances, contain the individual's DNA. Following from Locard's exchange principle, offenders of a crime will leave some form of biological material, specifically their DNA, at the crime scene. In crimes against another person (the victim), that person too will leave some DNA at the crime scene, creating the possibility that two sets of DNA will be found together, either because of the activity involved in the crime or due to chance cross-contamination of material from the victim and offender, whether the events are linked or not. The types of biological material that may be left by a perpetrator and/or victim include blood (white cells; red cells are anucleated), semen, hair roots, squamous epithelial cells (from saliva, vaginal fluid or urine) and endothelial cells (endocervical cells in vaginal fluid, urine or faeces).

Not all squamous epithelial cells (i.e., skin cells) contain DNA, although most do. Squamous epithelial cells with their DNA deposits may be found in the crime scene if a person has been kissed or licked by someone or something, or on cigarettes butts, drinking glasses or cutlery and in saliva deposits on materials such as clothing and handkerchiefs. These cells may also be found on the penis of a sexual offender who has either vaginally or orally penetrated a victim (Syndercombe Court, 2011).

Squamous epithelium of the skin, unlike squamous epithelium lining cavities (mouth, oesophagus, vagina, urethra and anus), is transformed from the nucleated basal layer cells to a keratinized, anucleated covering layer, called the stratum corneum. It is the stratum corneum that is regularly desquamated, leaving trace amounts of skin flakes at the crime scene. Although these desquamated cells cannot be expected to contain DNA, DNA profiling has shown that an individual's genetic profile can be retrieved from objects that they may have touched (Van Oorshot and Jones, 1997; Balogh, Burger, Bender et al., 2003). Initially, transferred DNA found on touched objects was thought to have originated from epidermal cells sloughed off the surface and carried onto the secondary surface by sweat. Kita and co-workers

(2008) undertook a morphological and immunohistochemical investigation of nuclear DNA in differentiating squamous cells in the skin and also a genetic analysis of DNA on swabs of human skin. They showed that single-stranded DNA was found in both the cornified layer of the skin and in swabs used to swab the skin. Further, they demonstrated the presence of small DNA fragments in those swabs and were of the opinion that the fragments on touched objects may originate from these normally believed to be anucleated keratinized cells. This nucleus-free, or cell-free, nucleic acid (CNA) has been shown to form a significant proportion of DNA found in sweat (Quinones and Daniel, 2012).

DNA can be deposited by a person touching any object with an uncovered part of their body and from deposited excretions (e.g., endothelial cells from sweat glands, vaginal fluid, saliva, faeces, semen). Flakes of keratinized skin are regularly shed and can be deposited on any surface. At a crime scene, DNA-containing cells and CNA may be deposited by the offender and victim, observers and investigators. As the victim and offender move to other locations (this includes making contact with law enforcement agencies and other persons), both may acquire and deposit (contaminate) their own and secondary DNA from a crime scene at these new locations, among which is the clinical examination suite.

The equipment and reagents used during the clinical examination to collect DNA specimens and by the laboratory to analyse the specimens may also be contaminated during the production, packaging, transport and storage phases (Archer, Hopwood and Rowlands, 2010; Gill, Rowlands, Bastisch et al., 2010, Goray, van Oorschot, Mitchell et al., 2012). International standards that seek to support this possibility are already in development (ISO/DIS 18385, 2014) and intend to set standards for ‘forensic DNA grade’ products where any DNA present would be below currently available limits of DNA detection. Under standard detection conditions this may be as low as 1.67 picograms (pg) (a single cell contains about 6pg DNA), and even lower where enhanced detection processes are in place (Promega Corporation, 2013). Minimization of contamination and cross-contamination is, therefore, essential when managing crime scenes, including the ‘human crime scene’ – both the victim and offender.

The potential sources of contamination and cross-contamination of DNA are numerous and present at all the steps in the investigation, from the crime scene to clinical examination suite and the laboratory. It is the clinical examination suite that is the principal consideration of this chapter.

Decontamination

With the ever-increasing sensitivity of DNA profiling, appropriate measures need to be taken at all steps of the DNA process to ensure minimization of contamination and cross-contamination (to be referred to collectively as contamination). The reason for this, as illustrated below, is that the amount of DNA from a crime scene is usually minute and may be poorly preserved. PCR will amplify the few molecules present, and any contaminating DNA may become a serious problem as low copy contamination will be amplified, leading to potentially misleading false-positive results. Within the clinical setting, therefore, appropriate use of decontamination

of the areas where a physical examination is to be undertaken and of the equipment used needs to be implemented. In selecting such decontamination measures, however, a number of issues will modify the ideal, theoretical approach taken. These include:

1. The efficiency of the decontamination technique. In considering the efficiency of the reagent used, the decontaminating reagent must be effective against cellular DNA and not just extracted or native DNA. Although some reagents may be effective in the denaturation of pure extracted or native DNA, they are not effective if the DNA is protected by cell and cell membranes (Schwark, Poetsch, Preusse-Prange et al., 2012).
2. Occupational health and safety considerations for the clinicians, nurses and cleaning staff as well as the victim and offender, both of whom are patients.
3. The effects of the use of the reagent on the efficiency and sensitivity of the PCR laboratory process.
4. The corrosiveness of the decontaminating reagents used in relation to the equipment within the examination suite, such as metal trolleys, colposcopes and cameras.
5. The effect on the doctor–patient and nurse–patient relationship.
6. The effect on the patient. Although the victim and offender may be ‘crime scenes’ they remain patients and should be accorded professional, non-judgemental care. The forensic examination should not add significant trauma to the crime that may have been committed; this is particularly true for paediatric cases.

Decontamination – the science

The DNA molecule and its stability

Deoxyribonucleic acid (DNA) is a long molecule, organized within cells in chromosomes. The human cell contains 23 pairs of chromosome, 22 of which are autosomes, plus two sex chromosomes. It is principally the autosomes that are examined to provide the information for a DNA profile.

The DNA molecule within the cell needs to be highly compacted. Specific proteins (histones) interact with DNA in nucleosomes, condensing to form a nucleoprotein complex (chromatin), held together by electrostatic forces, which can be broken down with high salt concentrations. Histones are not the only components that stabilize DNA within the chromosome which are, in addition, protected from the outside environment by the double cell wall, surrounding cytoplasm and cell membrane. DNA that has been removed (extracted) from its cellular surroundings will, therefore, be more vulnerable to agents that can fragment it.

Each DNA molecule consists of two strands coiled around one another to form a double helix. Each strand is composed of a long chain of nucleotide subunits. Each subunit has three components:

1. A phosphate group, which gives the molecule a negative charge, but which also contributes to instability in the double helix as the negatively charged groups repel each other, attached through phosphodiesterase bonds to:
2. A five-carbon ring (pentose) deoxyribose sugar at the third carbon below and the fifth carbon above; together these form the sugar-phosphate backbone. Attached to carbon 1 is:
3. A nitrogenous base. These bases are of two types: the purines – adenine (A) and guanine (G); and the pyrimidines – cytosine (C) and thymine (T).

The asymmetry of the phosphodiesterase bonds infers a direction to each strand, which is in an opposite direction (anti-parallel) to the other in the double helix formation. The double helix is stabilized by stacking of the hydrophobic nitrogenous bases within the molecule and through hydrogen bonds that form between the bases; two hydrogen bonds form between A and T bases, and three hydrogen bonds link G and C bases, providing added stability to areas of the DNA molecule that is 'GC-rich'.

Each nucleotide pair is known as a base pair (bp), and this notation is used as a measure of the length of a particular DNA strand sequence. The lengths of DNA sequences examined within a DNA profile is typically between 100 and 500 bp. Thus, any event that fragments DNA strands into sequences that are less than 100 bp in length cannot be profiled and, unless the larger fragments remain intact, any DNA profile may be incomplete (partial).

DNA can be damaged in two main ways: through mechanisms that break the hydrogen bonding between the complementary strands, destroying the double helix (DNA denaturation), and by mechanisms that break the sugar phosphate backbone (DNA damage) producing single- and double-stranded breaks, or through other interactions with the molecule, which distort the molecule or remove the bases. It is these latter damaging mechanisms that are more important when wanting to destroy (decontaminate) DNA, shortening or damaging the template molecule so that it cannot be amplified to produce a profile. Separation of the molecule into two strands through denaturation can also increase the vulnerability of the molecule to backbone damage.

DNA denaturation

Hydrogen bonds are relatively weak, allowing the two strands to be pulled apart. This is a necessary process in DNA replication, but the strands can also be separated by mechanical means and by heat. The temperature at which this latter takes place is known as the melting temperature. Three main factors affect this temperature: the GC content, because it is harder to break three hydrogen bonds than two; the salt content, because Na^+ ions interact with the negatively charged phosphate ions in the

DNA backbone, adding stability by shielding the charge repulsion between the backbone molecules; and the length of the molecule, because the more hydrogen bonds to be broken, the more difficult it is to separate the two strands easily.

Chemical denaturants can also break this hydrogen bonding; there are three main mechanisms. Chemicals that raise the pH (e.g., sodium hydroxide) will pull the hydrogen ions away from the nitrogenous base, thus removing the ability for the two strands to bond. Competitive denaturation can occur with some chemicals (e.g., urea and formaldehyde) as these compete with hydrogen ions to bond with the electronegative nitrogenous bases, particularly at high chemical concentrations. Some aldehydes (e.g., formaldehyde) can also form covalent bonds with the electronegative nitrogenous bases and block the formation of hydrogen bonds between complementary strands.

DNA damage

DNA, even if protected within a cell, can still be damaged by many different physical or chemical agents. While some are endogenous, formed inside the cell through normal metabolic pathways, others are exogenous, coming from the external environment. This damage may be introduced unintentionally or intentionally. It is for the latter purpose (decontamination) that particular agents are considered next.

Decontaminants

A limited number of published studies have been undertaken to determine the most effective means of decontaminating surfaces, equipment and reagents that may be used to collect and analyse biological specimens. To clarify this from the outset, water, 70% ethanol and detergents, although partly effective in cleaning away native and cellular DNA, do not decontaminate surfaces sufficiently for PCR application (Champlot, Berthelot, Pruvost et al., 2010). In addition, water and some detergents may contain cellular DNA and CNAs. The main decontaminants considered have been radiation (UV, beta and gamma), ethylene oxide, bleach, autoclaving and various commercial compounds. The latter will not be addressed in this chapter.

Experience indicates that there is some confusion between the concept of sterilization and DNA decontamination. Too many forensic practitioners equate sterilization with DNA decontamination. Although the same methods may be used for both, the process to achieve DNA decontamination is very different from that of sterilization. To clarify these differences, the procedures that apply to both sterilization and DNA decontamination, and principally cellular DNA decontamination, will be considered. A summary of the types of sterilization agents and DNA decontaminants are given in Table 1.1.

UV radiation

The use of UV radiation in the sterilization processes is limited (for an overview, see Lambert, 2013) due to its poor penetrative power, extensive absorption by glass and plastics and the very high radiation levels required. It has a greater application in disinfection than in sterilization procedures. UVA (400–320 nm wavelength) produces indirect damage through the production of free radicals. UVB (320–290 nm)

Table 1.1 Summary and comparison of sterilization and effective cellular DNA decontamination.

Surfaces (e.g., trolleys, couches, colposcopes, cameras)		
Method	Sterilization	DNA decontamination
UV light (254 nm)	Disinfection rather than sterilization	A useful adjunct to decontamination, but efficiency is poor and times for decontamination long and directly related to distance from the light source
Beta radiation	Unsuitable	Unsuitable
Gamma radiation	Unsuitable	Unsuitable
Ethylene oxide	Unsuitable	Unsuitable
Sodium hypochlorite	Disinfection only	3% (w/v) for 15 minutes Highly corrosive
Autoclave	Unsuitable	Unsuitable
Instruments and equipment		
Method	Sterilization	DNA decontamination
UV light	Unsuitable	Unsuitable
Beta radiation	25 kGy	In excess of 50–60 kGy (optimal dose yet to be determined)
Gama radiation	25 kGy	In excess of 50–60 kGy (optimal dose yet to be determined)
Ethylene oxide	30 min–10 hrs	6–12 hrs (optimal exposure conditions yet to be determined) Does not affect the DNA profiling process
Sodium hypochlorite	Disinfection only	3% for 15 minutes Highly corrosive
Autoclave	121–134 °C at a pressure of 15–30 psi for 3–15 minutes Not suitable for heat-sensitive materials and instruments	121 °C at a pressure of 15 psi for 120 minutes Not suitable for heat-sensitive materials and instruments

and UVC (280–100 nm) cause direct damage, forming pyrimidine–pyrimidine photo adducts (cross-links) between adjacent bases, leading to cyclobutyl pyrimidine dimers, oxidization of bases and the introduction of single- and double-strand breaks. The cross-linking alters and weakens the helical structure and can also inhibit the PCR amplification of these molecules (Champlot, Berthelot, Pruvost et al., 2011).

The use of UV light of 254 nm has been found to be effective in only very limited conditions for DNA decontamination. Hall and Ballantyne (2004) tested the effect of UV light on three DNA samples. They used a solution of extracted DNA, dehydrated extracted DNA and dried bloodstains. They found that it took 16 minutes of

treatment of the solution of extracted DNA in a UV cross-linker to prevent DNA amplification. By comparison, the dehydrated extracted DNA took 24 hours and the dried blood 102 hours to achieve the same outcome. Apart from UV radiation ineffectiveness for cellular DNA, their findings also illustrated the differences in susceptibility between native or extracted DNA and cellular DNA. It is the DNA within the cell that is very relevant to the forensic clinical practitioner.

Shaw and co-workers (2008) trialled UV irradiation in a cross-linker using saliva specimens. Irradiation of specimens for ten minutes was unsuccessful in denaturing the DNA samples. Gefrides and co-workers (2010) similarly trialled UV irradiation with dried saliva specimens. Irradiating the specimens for 60, 120 and 180 minutes they were still able to detect amplifiable DNA products at all times after UV treatment. From the studies undertaken, it was of note that the effectiveness of UV light decreases with the square of the distance between the UV light source and the irradiated item (Tamariz Voynarovska, Prinz and Caragine, 2006; Gefrides, Powell, Donley and Kahn, 2010; Champlot, Berthelot, Pruvost et al., 2011). Decontamination with UV light, therefore, would need to be restricted to surfaces that are relatively flat and close to the UV bulb. UV irradiation was found to be inefficient alone for decontamination of entire working areas and of laboratory and, by extension, clinical equipment (Tamariz, Voynarovska, Prinz and Caragine, 2006; Shaw Sesardic, Bristol et al., 2008; Champlot, Berthelot, Pruvost et al., 2011).

Beta and gamma radiation

Ionizing radiation, and in particular gamma and beta radiation, is widely used for the sterilization of medical products, especially thermolabile products (for an overview, see Lambert, 2013). Its application to surfaces and equipment, however, is not available in the clinical setting and any item to be treated with this form of radiation needs to be sent to an appropriately accredited facility. Gamma radiation is generally produced by cobalt-60 or caesium-137 radioactive sources, and beta radiation by high-speed electron beams. The former consists of electromagnetic radiation of short wavelength but with high energy and penetrating power. The latter consists of high-speed electrons with significant but less penetrating power than gamma radiation. The usual sterilizing dose is 25 kGy, although this may vary depending on the micro-organism type and load. Ionizing irradiation generates different lesions, either through direct ionization of DNA and enzymes or by the activity of free radicals in the cellular fluid. Double-stranded breaks in an area required to be amplified to provide a DNA profile will disable the process.

There is only one paper that addresses the use of beta and gamma radiation in the decontamination of cellular DNA. Shaw and co-workers (2008) trialled beta and gamma radiation on saliva samples at doses of 50 kGy and 56.4 kGy, respectively. DNA profiles were present in all samples after gamma radiation and in most samples after beta radiation. The research team was of the view that, under the conditions of their study, although both sources of radiation were able to reduce the number of full DNA profiles, this did not happen consistently. Currently, optimal conditions for beta and gamma radiation in DNA decontamination are yet to be determined.

Ethylene oxide

Ethylene oxide is a well-known sterilizing agent, particularly for medical equipment. It is an alkylating agent which is thought to inactivate microorganisms through the denaturation of nucleic acids and enzymes (for a review, see Mendes, Brandão and Silva, 2007; Dusseau, Duroselle and Freney, 2013). In the process, alkyl groups bind to sulfhydryl, hydroxyl, amino and carboxyl groups of proteins and purine bases of nucleic acids to form adducts, which leads to mispairing during DNA replication and results in single strand breaks. These adducts can also result in bases being lost (abasic sites), weakening the structure. For these reasons, ethylene oxide is potentially unsafe to those exposed to it. During the sterilization of some medical equipment, secondary toxic residues (e.g., ethylene chlorohydrin and ethylene glycol) may be formed. Ethylene oxide has been classified by the International Cancer Research Agency as a carcinogen due to observed genetic mutations in hospital staff accidentally exposed to the agent. Acute exposure at low concentration may cause allergic reactions, headaches, dizziness, nausea and irritation of the eyes and respiratory mucosa. Higher concentrations may result in central nervous system depression. Processes have been developed, however, to minimize residue exposure.

Ethylene oxide is used as a pure gas or is mixed with a gas carrier. Use as a pure gas requires care as ethylene oxide is highly flammable and explosive. In hospitals, it may be used in small cartridge forms to overcome these problems. Irrespective of whether it is used as a pure gas or with a gas carrier, there are certain parameters that must be optimized to ensure effective sterilization (Dusseau, Duroselle and Freney, 2013). These include:

- Relative humidity. The optimal humidity is 35% but it may be used between 40% and 80% for adequate penetration of the items and to assist the alkylation process.
- Gas concentration. This is usually between 400 mg/l and 1,000 mg/l. Higher concentrations may be used, but these increase toxicity and desorption times.
- Temperature. This is usually 40–50 °C.
- Duration. This varies from 30 minutes to 10 hours.

A desorption cycle is required and may last for hours or weeks.

For the purposes of DNA decontamination, Shaw and co-workers (2008) conducted a series of experiments using ethylene oxide for different durations on various volumes of saliva to test its effectiveness in denaturing the DNA and to observe whether the ethylene oxide inhibited DNA analysis. The gas concentration and parameters, other than exposure times, were not stated in the paper. Of the samples treated with ethylene oxide for four hours, no DNA profiles of sufficient quality for loading onto the UK DNA database were obtained and 13% of all samples did not produce any DNA profile. After treatment for six hours, no sample had a DNA concentration at a level for effective profiling. However, current techniques are more sensitive and appropriate exposure times may need to be reconsidered. Treating

cotton swabs with the ethylene oxide decreased the amount of DNA on the swab by 99.98%. Importantly, ethylene oxide treatment of equipment did not appear to have any adverse effect on DNA profiling.

Archer and colleagues (2010) examined the optimal ethylene oxide exposure to remove DNA from disposable consumables after spiking the consumables (cotton swabs and stain cards) with blood, saliva and DNA by touch from a volunteer who had a high propensity to deposit DNA. Ethylene oxide cycles (1 cycle = 6 hours' exposure) varied between 0 and 2 with exposure times of 1–12 hours. After half a cycle (3 hours' exposure), the percentage profiles recovered from the stain cards and swabs was 8% and 13.2%, respectively. After two cycles (12 hours' exposure), the recovered profiles fell to 0.4% for both sample types.

Archer and colleagues extended their studies to observe the effect of two ethylene oxide cycles on excessive DNA loads from saliva, blood and semen on swabs. The two cycles yielded few profiles but semen stains proved more resistant. Like Shaw and co-workers (2008), they did not find that treatment of the swabs affected the PCR process.

Discussions with colleagues would query the effect of ethylene oxide on the PCR process. There are unpublished claims that the process may be inhibited by pre-treating certain swabs with ethylene oxide. This suggestion is not, however, supported by the published data: Shaw and colleagues (2008) undertook a study of tubes and swabs, comparing DNA profiles obtained from those sterilized with ethylene oxide and those not sterilized, and found no difference. Absent from both the published and non-published data is the full detail of the ethylene oxide treatment used; it is, therefore, difficult to compare procedures and ascertain the minimum decontamination process that is required clinically. Certainly, exposure for 6–12 hours may be required to decontaminate swabs and other equipment used in the clinical setting successfully.

Like beta and gamma radiation, a disadvantage of using ethylene oxide is that treatment of any material needs to be undertaken away from the clinical setting. Trolleys, examination couches and other surfaces cannot be decontaminated in this way. There is also an occupational health and safety issue, discussed briefly above. One of the advantages, however, by comparison with sodium hypochlorite, is that it is not corrosive to metals and so can be used with many pieces of medical equipment.

Sodium hypochlorite (bleach)

Sodium hypochlorite is an inexpensive, effective disinfectant and bleaching agent, though not a sterilizing agent, and is readily available as household liquid bleach in concentrations of 5–10%. It is a strong oxidizing agent that liberates toxic gases (e.g., chlorine) when acidified or heated (for an overview, see IPCS, WHO). When mixed with ammonia or with substances that can generate ammonia, sodium hypochlorite can produce chloramines, which are also toxic and have an explosive potential. It can also produce carcinogens when combined with chemicals such as formaldehyde. Both chlorine gas and chloramines are strong respiratory irritants and chlorine gas becomes a problem when it is mixed with acids. The resultant chlorine gas may cause immediate eye, nose and respiratory irritation, chest tightness, coughing, wheezing and dyspnoea. As sodium hypochlorite is corrosive, contact with skin may cause burning pain, inflammation and blistering. In relation to DNA, sodium hypochlorite, as a strong oxidizing agent, causes base modifications, the

formation of chlorinated base products and, with increasing concentration, strand breakage and the formation of individual bases (Kemp and Smith, 2005). Some formulations may need daily preparation in order to maintain efficacy.

Due to the lack of consensus on the optimal treatment of surfaces to eliminate contaminating DNA, Kemp and Smith (2005) reassessed the optimal concentration and time needed to remove all contaminating DNA from the surfaces of teeth and bones. Similar reports had suggested concentrations of 5–20% and exposure times of 1–20 minutes, respectively. Using ancient bone samples, one of the authors handled the bone for 30 minutes to provide contamination, and testing was undertaken to identify his mitochondrial DNA using different sodium hypochlorite concentrations and exposure times. They showed that bleach at a concentration of at least 3% (w/v) and immersion for at least 15 minutes was required for successful surface decontamination of DNA. Champlot and colleagues (2010) also looked at the effectiveness of objects wiped with hypochlorite solution (solution of 2.6% active chlorine). In their study, they found that bleach, by wiping, rinsing and soaking, was the most effective means. Unfortunately, the time of exposure to sodium hypochlorite was not disclosed – the researchers refer simply to ‘generous treatment with bleach as being effective’.

Unlike Kemp and Smith (2005), Schwark and colleagues (2012) assessed forceps and autopsy tables for DNA contamination after treatment with bleach (DAN Klorix®, Colgate Palmolive, Vienna, Austria) at concentrations of 2.8% and 0.28%. They found that soaking of forceps initially cleaned with soap and water, and scrubbing and soaking tables with both concentrations of bleach for 30 and 15 minutes, respectively, followed by rinsing with water successfully removed all DNA contamination. Unfortunately, they did not specify the content of the bleach solution and a search of the Internet was unrevealing. The applicability of Schwark and colleagues’ (2012) method to clinical practice is difficult to evaluate and implement for trolleys and other surfaces, but it may point to a possible means of effectively cleaning and decontaminating some instruments used in the clinical suite.

Autoclaving

Heat is known to inactivate life forms and, in combination with steam, is a very effective means of sterilization. Aqueous solutions are subject to DNA oxidation and acid hydrolysis damage; heating will speed these processes. The steam autoclave is one of the oldest forms of sterilization. The process involves packaging the items to be sterilized and placing them in a sealed chamber where steam reaches 121–134 °C at pressures of 15–30 psi (103–207 kPa). The period of sterilization varies with the size of load and may last for 10–60 minutes (for an overview, see Hancock, 2013). For most medical applications, the standard autoclaving process is 121 °C at 15 psi for 15 minutes. Although the process is relatively safe, effective, economical, reliable and fast, it exposes materials to heat and moisture. Therefore, it is not applicable in all circumstances.

Gefrides and colleagues (2010) observed the effects of autoclaving on DNA contamination. They used dry and fresh saliva samples in microcentrifuge tubes subjected to autoclaving at 121 °C at 15 psi for 0–180 minutes. The tubes were autoclaved in three configurations – uncapped, capped and uncapped but within a sterilization pouch. Not surprisingly, the number of detectable alleles decreased with increasing autoclave duration. For those samples exposed to steam (i.e., uncapped and not in a

sterilization pouch), complete decontamination was reached at 120 minutes of sterilization (i.e., 2×60 minute cycles) but not at 60 minutes. Even at 120 minutes, the sample in the capped tube still showed alleles, indicating that for DNA decontamination to occur, direct exposure of the DNA to steam is essential. The success of decontamination was also volume-dependent. For fresh saliva samples, autoclaving a 10 μ L undiluted sample and a 50 μ L fresh diluted and undiluted sample showed an absence of alleles in the 10 μ L sample at 120 minutes but residual alleles in both the 50 μ L samples.

General approach to DNA decontamination

Given the potential outcome of criminal court cases in which DNA evidence may be tendered and the propensity for contamination to occur, the very limited number of published investigations into the most suitable system of minimizing decontamination especially in the clinical setting is most disconcerting. Overall, the most effective means of decontamination of medical equipment for use in the collection of biological samples is the use of ethylene oxide. For those areas where it is not possible to utilize ethylene oxide, sodium hypochlorite at a concentration of 3% or higher is required, with a period of immersion of 15 minutes or more. Some formulations may need to be prepared daily in order to maintain efficacy. Spraying and immediately wiping of surfaces with sodium hypochlorite is not fully effective. Sodium hypochlorite is not suitable for use on many pieces of equipment used in the clinical examination room, including colposcopes and cameras. Metal trolleys that are used as benches and for other purposes within these examination rooms are susceptible to the corrosive effect of this highly oxidizing agent. Although optimal conditions for DNA decontamination are evolving with the increasing sensitivity of the PCR process, there remains a dearth of evidence-based criteria.

Contamination

The approach to biological specimen collection for the purposes of DNA analysis varies among jurisdictions. It may range from specimens being collected by police officers or police-employed scientists to the engagement of forensic practitioners (nurses, doctors, paramedics). The examinations may be undertaken in a variety of locations, but may involve dedicated examination suites often attached to a major hospital. Examinations in those suites may be limited to alleged victims, although in some suites examination of both the victim and offender may occur. The attending police officer, scientist or forensic practitioner may be required to wear protective clothing, head and facial covering and gloves, and the areas where alleged victims and offenders are examined may be required to be cleaned prior to use.

Whatever process is in place, the examination of living humans can complicate the manner in which specimens are collected. Alleged victims and offenders are effectively crime scenes, but as such they are crime scenes with special needs. Potential victims of alleged crimes may have been traumatized by the crime and it is important that any subsequent forensic investigation does not exacerbate their trauma. This is particularly the case with children. Although the science discussed above is clear in

an ideal situation, managing a live human in a clinical setting may require modification of the approach to that person and this modification may increase the potential for DNA contamination to occur. Processes have been put in place in various jurisdictions to minimize the potential for contamination. In many cases, the approach is pragmatic and appropriate; in others, the approach and procedures utilized indicate a lack of understanding of the science of DNA.

The non-science

Let us consider briefly some of the non-science that exists. Included are the following:

- **Sterilization is the same as DNA decontamination.** Sterilization will kill or destroy microorganisms and damage the DNA. The agents used for sterilization will not, however, under the conditions required for sterilization, denature DNA to the extent necessary to prevent detection of contaminating alleles following the PCR process. To remove DNA sufficiently to exclude contaminating alleles, the conditions of treatment with sterilizing agents need to be modified. It is not, therefore, satisfactory to assume that because the item is sterile it is free of contaminating DNA. When choosing a provider of DNA-free equipment and reagents, certainty of the success of the process is essential. All suppliers should be expected to have batch controls to illustrate that their system has worked on each and every occasion, and that their process does not inhibit the subsequent PCR process.
- **The surface is DNA-free after spraying and wiping with sodium hypochlorite.** Although this method may remove some DNA, it does not decontaminate the surface. Research to date suggests that irrigation and exposure to approximately 3% sodium hypochlorite solution for an extended period (15 minutes) is efficacious, but this is almost impossible to achieve in standard laboratory settings and practical and effective approaches are yet to be defined.
- **Gowns, masks and gloves will prevent DNA contamination.** In most clinical settings, however, neither the gowns nor the gloves will be DNA-free, even after sterilization. Their use may reduce the extent of DNA contamination, but there is no evidence for this. Use of gowns may reduce the spread of contaminating DNA from street clothing to the working areas in the examination room, but that is all. Confinement of protective clothing to a particular working environment can also assist in limiting transfer from other areas.
- **Forensic clean rooms, where a ‘forensic clean’ will clear the room of contaminating DNA.** Various jurisdictions recommend dedicated rooms for the examination of victims and a separate dedicated room for offenders (this is also attempted when examining items from these individuals within a laboratory setting). The reasoning behind this is logical, but dedicated facilities are not available in all locations. Dedicated rooms may also allow restricted access (and the extent of contaminating DNA) and enable opportunities for a ‘forensic

clean', whatever that means. DNA laboratories are expected to undertake an ongoing, before-and-after case cleaning procedure that is monitored and shown to be effective. Sometimes it will be necessary to deep clean a facility and this should be done by agencies that understand the purpose and efficiency of the processes they use. Ideally, an agency performing this task should be ISO-accredited. Observation of the 'forensically cleaned' concept in practice can be an education in the absence of the above considerations and the following example (observed by one of the authors, J.G.) illustrates what may be provided by a contracted agency.

In one location, a large tertiary hospital, the cleaners arrived to 'forensically clean' the examination suite following a forensic examination. Dressed in the clothing they had been wearing around the hospital that day, the cleaners arrived pushing their cleaning trolley laden with a multitude of cleaning implements and solutions. One was pushing a bucket on wheels, in which was a mop, the handle of the mop being used to direct the bucket, and some fluid. They proceeded to clean the room, wiping the surfaces with various pieces of cloth obtained from a container with other cloths, using various sprays and eventually mopping the floor with whatever fluid was in the bucket. Gloves were worn for the cleaning process but no over-clothes or gown. Within five minutes or less, the 'forensic clean' had been completed and the log filled in. The cleaners then left with their equipment for the next call within the hospital.

The obvious questions to be asked are: Where had they been before they undertook the 'forensic clean'? What DNA-contaminating clean had they done before arriving in the forensic area? What vomit, blood and other biological spill had the cloths, mop and bucket content been used to clean away just before 'forensically cleaning' the forensic examination suite? Were the mops, cloths and bucket thoroughly cleaned and decontaminated before entering the forensic room on each and every occasion in order to remove the potential DNA load? The answer to all these questions is presumably 'no'. If the answer is 'no', then why pretend that the forensic room has received anything other than a standard clean and that somehow this 'forensic clean' is better than a standard clean, with the potential outcome that the 'clean' may simply have made the environment dirtier than it was before?

Cleaning and the DNA laboratory

Cleaning a DNA laboratory and appropriate disposal of used consumables in clinical waste are vital to reduce the risk of cross-contamination, which can significantly influence the outcome of a case, as we have seen. Transfer of cellular material that can be detected within the sensitive DNA profiling methods used will occur unless processes are in place to prevent or at least minimize transfer, or to remove any transferred material before it contaminates other items that may be examined for the presence of DNA.

The International Laboratory Accreditation Co-operation (ILAC) has published guidelines for forensic science laboratories (ILAC, 2012). These offer general guidance relating to laboratories involved in the analysis of trace-level materials, such as DNA. They include the need for physical separation of areas for high- (DNA) level and low-level work which are restricted to essential personnel, and the need for

these areas to be carefully controlled and appropriate records kept. The guidelines have not been updated, but individual jurisdictions and laboratories will be expected to have schedules within their ISO accreditation that detail the appropriate procedures, which will include environmental control and monitoring processes. They will also include measures to clean and decontaminate a laboratory area and to assess the efficacy of these processes. Some cleaning materials will also be able (understandably) to inhibit the required amplification of evidential material for DNA analysis and measures must be employed to minimize this inhibition when a cleaning agent is selected.

Good laboratory practice

General practice within a DNA laboratory will include:

- Working in areas which have a positive pressure environment to minimize CNA inflow. Where laboratories have not been purpose-built, many organizations will use positive airflow cabinets. Many of these cabinets will include the possibility of having UV irradiation of the area.
- Wearing a 'Howie'-style laboratory coat with its mandarin collar and elasticated wrists, designed to minimize transfer of biologicals or chemicals to street clothes. The use of a particular coat in a DNA setting must be restricted to a particular area of the laboratory. Some laboratories will colour-code coats for this purpose, or they may use disposable coats to be used only in a particular area. This is especially important when a scientist moves between post-amplification areas, where DNA is present at very high levels, and pre-amplification areas. Some laboratories may even restrict staff movement between these areas during any one day, or require full showers to be taken and the removal of outside clothing.
- No items should ever be moved from a post-amplification area to a pre-amplification area. The laboratory should ensure a one-way flow for all samples with dedicated equipment, reagents and protective clothing. Separate rooms will be necessary for reagent preparation, storage and office work, as necessary. Post-amplification material should be frozen and stored in a separate area.
- Wearing gloves which, at a minimum, must be replaced after each step of a process, or if they become contaminated. Better practice is to double-glove, wiping the outer surfaces with an antibacterial wipe, allowing safe degloving between processes. The gloves must cover the skin over the wrist at all times. The use of long wrist gloves, or taping, to ensure this is commonplace.
- Facial coverings in the form of disposable mob-caps, masks and laboratory glasses will help prevent contamination of the area from the analyst, as will a policy to limit conversation within the examination room.

- Provision of a 'gowning' area, or ante-room with hand-washing facilities, is a good approach to examination rooms. Gowning order can also be important. In situations where it is relevant to cover the face and hair (necessary in some laboratory settings when examining what might be very low-level DNA), the order is important. Applying a mask and mob-cap after putting on gloves can lead to the latter being contaminated. Similarly, gloves must be put on before the gown, in order to avoid contaminating the outside of the gown. The movement of paperwork should be limited and never allowed between a post-amplification and a pre-amplification area. Printing to areas outside the laboratory from dedicated computers within the area can assist in this.
- A regular daily or weekly (as appropriate) separate cleaning regime, recorded and monitored with environmental DNA swabbing of designated areas.

Work surfaces and equipment

- All work surfaces must be cleaned before and after contact with each evidential item. This should be recorded.
- Surfaces should be cleaned with a bleach solution (5.25% hypochlorite is often recommended) and then rinsed with pure water or alcohol to prevent build-up of sodium hypochlorite crystals. This is particularly important to minimize the corrosive effect of hypochlorite.
- Although flooding a surface with the fluid and leaving this for a period of minutes might be preferable, this may be very difficult to achieve in a busy laboratory, certainly if this is to be done between item examination in any case. Minimization of carry-over may be achieved by instituting a double cleaning process and the use of surface covering, as described below.
- Surfaces may be given added protection by using Benchkote or other smooth and water-resistant heavy paper (e.g., glassine paper). The paper is changed between each item examined and the surface cleaned as above.
- Equipment such as centrifuges, racks, pipettes and small instruments should be cleaned before and after use with the laboratory's selected surface wipe.
- Wherever possible, the use of disposable instruments (e.g., scalpels) should be employed and opened just prior to when they are required.

Samples and reagents

- Samples should be processed individually, only the sample being worked on at the time being exposed to the air and sealed immediately after.

- Wherever possible within a case, examination of items thought to carry less material should be prioritized so that heavily stained items are looked at later in the process.
- Tubes containing biological material, such as microcentrifuge tubes, should be centrifuged before opening. A tube opener or absorbent material barrier should be used to open the tube, rather than using gloved fingers.
- Fresh, aerosol-resistant pipette tips should be used for each sampling, tips also being disposed of if the pipette comes into contact with any surface other than the one intended (e.g., the bench), with appropriate cleaning to follow.
- Good manual processing within the laboratory will have a single flow direction for the analyst – pipette → pipette tip attachment → reagent → sample rack → tip waste container – so that movement does not produce aerosols that come back to contaminate any open tubes or items.
- Robotics are often used. It is the responsibility of the laboratories to ensure that procedures are proper and that contamination does not occur (see the Adam Scott case, above).
- Reference samples (from a known individual, likely to contain large amounts of DNA) should be processed separately, separated by time and/or space. Many laboratories have separate geographical facilities to process their reference samples.
- Separate areas should be reserved for examination of items from a victim or offender wherever possible. The areas used must be documented and preferably different scientists allocated to the examination of different sources of within-case items.

Laboratory decontamination

Sometimes it will be necessary to undertake a more thorough clean, and many of these practices can be brought into the regular daily or weekly clean of an area. These processes typically include:

- Surface flooding with a bleach solution left for 15 minutes before being wiped and cleansed with water or ethanol to eliminate the possibility of build-up or the introduction of bleach into a sample.
- Floor cleaning with a sodium hypochlorite solution.
- UV irradiation of areas, which can assist in removing extraneous DNA, along with other decontamination processes, and may be available in safety cabinets or small UV boxes. Small items (e.g., pipettes, tubes) can also be irradiated in

these cabinets. Exposure at 254nm is recommended for a minimum of five minutes; a 30-minute procedure is often built into laboratory protocols before and after use of hoods for PCR work, for example.

- Disposables should be sourced that are guaranteed as 'DNA-free' with a process that involved assessment of this using a PCR-based process.
- Laboratory coats should be laundered regularly, separating coats from different areas from the same wash.

Cleaning and decontamination of forensic examination suites – the way forward

As a result of the Vincent enquiry, modifications were made to the way in which forensic procedures were undertaken in the State of Victoria. Many of these recommendations were adopted and extended by the Australasian Association of Forensic Physicians (AAFP) (now the Faculty of Clinical Forensic Medicine of the Royal College of Pathologists of Australasia.) In 2013, the Association developed a recommendation for the cleaning and decontamination of forensic examination suites. A similar set of recommendations has been developed by the Faculty of Forensic and Legal Medicine (FFLM) of the Royal College of Physicians, London. These recommendations are available on the Internet (FFLM, 2012; AAFP, 2013). The AAFP recommendations are more detailed in respect of the cleaning and the general use of the forensic examination suite and will be used as a basis for discussion and recommendation.

The AAFP recommendation states that 'All areas used for forensic evidence collection should be cleaned for both infection control purposes and for decontamination of DNA.' The purposes of the recommendation are to 'provide a framework for both minimum level and best practice in cleaning, decontamination and prevention of contamination of forensic examination suites and it is important to 'demonstrate that processes to limit/prevent contamination as part of the forensic procedure' and 'to reassure the public and the judicial system that the risk of contamination is being managed/minimized'. The AAFP recommendation contains a series of principles which acknowledge that it is not possible to prevent DNA contamination totally; rather, the aim should be minimization of contamination and the ability to recognize contamination when it occurs. They also, appropriately, point out that it is important to maintain an environment that is welcoming to the patient and that is not totally 'sterile'. For example, rather than donning a face mask, which can make personnel look alien and frightening to some, the potential for contamination can be reduced by avoiding speaking while undertaking the examination. Equally importantly, they indicate that cleaning and decontamination procedures need to be safe and effective, and that procedures should be in place to ensure that any forensic specimen taken is done with the minimum risk of contamination. Importantly, offenders and victims should not be examined in the same suite.

With respect to a minimum cleaning protocol for the forensic examination suites, the AAFP recommend the daily cleaning of the floor and of the couch, bench tops, sinks and taps and writing desk, the weekly cleaning of other surfaces, the wearing of gloves, the use of protocols for cleaning and the keeping of a register of cleaning. The proposals seem sound. The AAFP recommendations then outline the minimum decontamination protocol for forensic examination suites. The first six statements read as follows:

1. The suite needs to be decontaminated before each use to prevent DNA contamination.
2. Decontamination entails the use of solutions that actively decontaminate DNA – for example, 0.5% sodium hypochlorite (bleach) (Det-Sol 500, Contain) or any disinfectant which is viricidal. Standard cleaning detergents do not decontaminate the DNA. Most decontaminating solutions need to be left on surfaces for 3–5 minutes. Decontamination solutions need to be wiped off using a dry white paper towel or dry cloth wipe such as Wypall, or may be followed by wiping with 70% ethanol wipes.
3. There are some detergent/DNA decontaminant combinations that could be used (e.g., Virachlor, Actichlor). These substances enable combined cleaning/DNA decontamination to occur.
4. All work surfaces, the examination couch, instruments, examination lights, cameras, writing instruments, trolleys, bench tops, writing desk, sink and taps need to be decontaminated before each use of the suite.
5. Alcohol-based wipes with organic content (e.g., Medi wipes) can be used to clean items such as scissors.
6. A register of completed decontamination should be maintained.

It is here that some problems arise. Although the concept of decontaminating a clinical examination suite may seem appropriate, there are some practical and occupational health and safety issues that make it almost impossible. Decontamination, as discussed above, is difficult with large areas and objects, and especially with sensitive equipment such as cameras and colposcopes. The most cost-effective means currently available for DNA decontamination is the use of sodium hypochlorite. From the available data, a solution of 3% is probably needed and this should be in contact with the instrument or surface for at least 15 minutes. Diluted sodium hypochlorite solutions are unstable and need to be prepared on a daily basis. Ethanol is not a DNA decontaminating agent and should not be used for this purpose. The suggested commercial products are discussed below.

The AAFP recommendations outline other ‘essential’ measures to reduce the likelihood of DNA contamination. These involve:

- The use of dedicated forensic suites.
- The need to have procedures in place to limit DNA contamination in non-forensic-dedicated facilities.

- The use of securely stored specific evidence collection kits.
- Discarding unused items in the evidence kits.
- Preferred use of disposable items/equipment.
- Use of dental wrap to cover difficult to decontaminate items such as colposcope handles.
- The changing of gloves before each step of the forensic process.
- Use of fresh linen and prompt removal of soiled linen and other waste.
- Appropriate training of practitioners.

The recommendations also comment on the need for dedicated examination suites, the use of log books documenting entry and use of the suite, and of the need for non-fabric floor and furniture coverings to enable appropriate cleaning. Finally, a list of measures to ‘evaluate the risk of contamination’ is provided, however this list is of logs, pro formas and registers does not address any risk of contamination. Although many of the AAFP’s final list of recommendations seems satisfactory, there are some questionable suggestions. These relate, in particular, to the use of gloves, linen and overclothing, the use of commercially available ‘decontamination’ solutions, and the whole concept of decontamination and its evaluation.

Gloves, linen and overclothing

Anecdotal evidence is that the wearing of gloves gives the wearer a false sense of security. Gloves in the forensic setting are designed to prevent the examiner’s skin cells and other DNA from contaminating the specimen being collected. They also have an infection control role. But the purpose of utilizing gloves is defeated if they are not used appropriately. ‘Appropriately’ means that the gloves should be used for the sole purpose for which they are being worn, namely, to protect the wearer and to prevent the transfer of their DNA to the specimen. Unfortunately, too often the wearers of gloves do not understand the principle of their wearing that pair of gloves. Take, for example, the scientist in the laboratory weighing out a chemical carcinogen. They enter the weighing room wearing gloves and carrying the bottle of carcinogen to a set of scales where they decant some of the specimen using a spatula onto a weighing dish and weigh the specimen. The purpose of wearing the gloves is to ensure that they are protected from the carcinogen. In weighing the carcinogen, they inadvertently touch the carcinogenic material with their gloves and, sequentially with their contaminated glove, touch the weighing device controls, then the outside of the carcinogen container as they replace the lid, and then leave the room, touching the door handle, and eventually put the carcinogen-containing bottle back on the shelf. They may then touch other items, including pens, computer keyboards, equipment, door handles, benches, and so on, spreading the carcinogen.

Degloved, they and others using the area will be exposed to any or all of those items and expose them to the risk of the carcinogen. The gloves have given a false sense of security to the scientist, who is subsequently placed at risk, as are many others. Similar situations occur within the forensic suite exposing a potential for DNA contamination.

Protective clothing is no different from gloves. Their purpose is to prevent the examiner's clothing contaminating the scene. It is not uncommon to see persons wearing full protective clothing, whether forensic or surgical, and being out and about in other areas remote from the surgical theatre or forensic suite, ensuring ongoing contamination. In essence, when gloves or overclothing are worn it should be worn solely for the purposes for which it is being worn and then removed to avoid cross-contamination and infection. It is not uncommon for some examiners to think that wearing gloves will prevent contamination, without considering that the gloves themselves can readily transfer material to other scenes.

Another consideration is whether the gloves, overclothing and linen are DNA-free. There is little point in decontaminating a couch or trolley of DNA and then placing a DNA-contaminated item on it in preparation for a forensic procedure. Washing and autoclaving using standard techniques do not denature all potentially contaminating DNA. Disposables bought from manufacturers are not necessarily DNA-free. Gloves, which are the most likely and frequently used item in forensic medicine to prevent contamination, may themselves be contaminated. Sterile surgical gloves are not necessarily DNA-free. Non-sterile boxes of multiply-packaged gloves are certainly not DNA-free, as Daniel and van Oorschot (2011) demonstrated.

Recalling what happened in relation to the 'Phantom of Heilbronn', whenever using a forensic specimen collection kit and particularly swabs, or any clothing and gloves, consideration needs to be given to whether these items were DNA-free prior to use or not. Any material that has not been appropriately treated by validated means may not be DNA-free.

Commercially available 'decontamination' solutions

Many commercially available products are available to clean surfaces and instruments prior to use in various medical and other procedures. Based on the available data for DNA decontamination processes, the choice of a suitable commercial DNA decontamination solution or system needs to be carefully considered when selecting it for use in the forensic setting. There are three primary considerations: 1) Does it denature cellular DNA, as opposed to native or extracted DNA, to a point where the alleles are too short for detection in the PCR process? 2) Does the compound or an additive have any inhibiting effect on the PCR process? 3) Is it safe to use in the clinical setting?

FFLM refers to one commercially available disinfectant: Virusolve®. This solution contains 2-aminoethanol, potassium carbonate and alkyl triamine plus surfactants. Although the product is sold as a disinfectant, published evidence is required to assess its suitability for DNA decontamination in the forensic system and to ensure that it does not inhibit the PCR process.

Decontamination and its evaluation

It is essential that any equipment used for the collection of DNA evidence should be DNA-free initially and the supplier needs to be certain that appropriate controls are run with every batch produced to confirm this. However, does the entire clinical examination suite or only specific working surfaces and equipment need to be DNA-clean? The authors' view is that the answer is 'no' to the former and 'yes' to the latter if the collection is undertaken competently. Working surfaces, whether a trolley, bench or a cover, must be decontaminated appropriately before use. Specimens should be collected with DNA-free equipment and properly sealed in dedicated packaging. The specimens should not come in contact with any potentially contaminating surface or piece of equipment. For instance, when swabbing a specimen, the swab should not be touched by the collector. It must be unsheathed immediately prior to use and then immediately resheathed. It should never be left open on surfaces to dry or pending labelling or other processes. Moist collections are more vulnerable to subsequent DNA degradation through microbial contamination. Consequently, emphasis has been placed on allowing swabs to dry before sealing the containers, but this has encouraged the practice of leaving swabs open and hence vulnerable to inadvertent contamination. Some swab containers are now available with active drying systems within the cap to overcome this problem. If the swab must come in contact with a surface, such as a microscope slide, then that surface, or at least the batch, must be known to be DNA-free, assessed through a PCR-based testing process.

One issue that the AAFP and FFLM recommendations do not consider is the order of collection of forensic specimens even though this may adversely affect contamination. The appropriate order of collection and the need to collect specimens at the earliest opportunity in any examination needs to be stressed. Similarly, it is important to ensure when collecting a specimen from one site that there is no possibility of cross-contaminating that site with another site. Consider the example of taking low and high vaginal swabs. It is important that the low vaginal swab is taken first so that any potential for contamination of a high vaginal swab can be determined, particularly in the case where DNA of an alleged offender is located on the low vaginal swab.

In some jurisdictions, early evidence kits (EEK) can be employed prior to a formal forensic examination when a complainant first reports to the police. In addition to a urine sample for drugs and alcohol, a second urine sample may collect useful drainage after a sexual assault. The kit also contains a mouth swab and rinse so that important forensic evidence is not lost. Specimens need to be taken before there is an opportunity for contamination of those specimen sites with extraneous DNA.

Overall, the recommendations of the AAFP and FFLM are similar and generally satisfactory, but with some notable areas of questionable advice as discussed above; the main issues relating to the principles of DNA decontamination. It is the authors' view that, in the clinical setting, absolute DNA decontamination is unachievable and that minimization is the goal. This requires a fall-back position, which may be adequately addressed by implementing appropriate DNA databases of forensic personnel similar to those that exist in some jurisdictions.

DNA elimination databases

Within the clinical setting, the reality is that DNA contamination cannot be fully excluded if the person being examined is to be treated in a manner that is unlikely to exacerbate or create a traumatic experience. Recommendations have been made that all clinical examiners and laboratory staff should have their DNA profile on an appropriate database so that any contaminating DNA from the clinician or scientist can be excluded in the DNA analysis. Manufacturers of consumables used for DNA evidence collection are also being encouraged to develop DNA elimination databases of key staff.

The inclusion of forensic practitioners' and forensic scientists' DNA on a database is not new and databases can be found in many jurisdictions, several of which are attempting to extend them to include relevant police personnel and manufacturers. Privacy issues can be addressed by: 1) the DNA sample being supplied without the donor's name attached to it; 2) the database being used exclusively by the laboratory for exclusion purposes only and not as part of a general criminal database; and 3) legislation being in place that penalizes any misuse of the DNA database.

Not all jurisdictions, however, have appropriate safeguards and any forensic practitioner asked to provide a sample needs to take this into account. Any forensic practitioner who provides a DNA sample should enter into a written agreement with the agency that tests the sample, and then interprets and reports the results. The agreement should provide assurances that: the DNA sample will not be used for any other purpose without the written consent of the donor; the sample and DNA profile should be stored securely and separately from samples collected from complainants, suspects or others involved in criminal investigations; the database should be independent and separate from criminal or other agency DNA databases; and penalties should be levied on any person or agency that misuses the forensic practitioner's sample or DNA profile.

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