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Reducing Contamination in Forensic Science

Abstract

The sensitivity of modern forensic techniques has drastically increased, with sensitive technology detecting even the smallest traces of DNA evidence left behind. This has made it possible to detect DNA profiles deposited through contamination. When DNA contamination occurs in forensic science, it has the potential to change the outcome of a criminal investigation and may have significant social and financial repercussions. A compilation of global research shows that DNA evidence transfer can occur during forensic product manufacturing, the fingerprinting process, or even autopsy and crime lab examinations. These vital areas of the forensic investigation are vulnerable to contamination, and national standards should address this susceptibility. Understanding the origins of contamination events provides the greatest insight into preventing their occurrence and maintaining the integrity of forensic evidence.

Keywords

forensic science, DNA profiles, contamination

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Abstract

The sensitivity of modern forensic techniques has drastically increased, with sensitive technology detecting even the smallest traces of DNA evidence left behind. This has made it possible to detect DNA profiles deposited through contamination. When DNA contamination occurs in forensic science, it has the potential to change the outcome of a criminal investigation and may have significant social and financial repercussions. A compilation of global research shows that DNA evidence transfer can occur during forensic product manufacturing, the fingerprinting process, or even autopsy and crime lab examinations. These vital areas of the forensic investigation are vulnerable to contamination, and national standards should address this susceptibility. Understanding the origins of contamination events provides the greatest insight into preventing their occurrence and maintaining the integrity of forensic evidence.

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Introduction

Locard's Exchange Principle states that every contact made will leave behind traces of evidence that can only be misinterpreted through human error (Goray, van Oorschot & Mitchell, 2012). In many cases, the most valuable traces are those that have biological origins, and from these samples a DNA profile can often be derived. The increasing sensitivity of forensic testing has made it possible to produce millions of copies of a DNA molecule within a sample; as few as 10 cells are needed for this technique, and some labs have reported successful amplification of single molecules (Hampikian, 2012). Such sensitivity brings with it the issue of amplifying samples that originate from contamination. In the context of forensic science, contamination can be considered to be any deposited material not relevant to the crime under investigation (van Oorschot, Ballantyne & Mitchell, 2010). Evidence contamination can incorrectly implicate or exonerate individuals, and make an exhibit useless for subsequent courtroom proceedings (Shaw, 2007). The presence of contamination requires further resources, including sterilization techniques and extensive interpretation of evidence to ensure accurate analysis. Contamination events revealed in the courtroom can discredit both the evidence and the competence of the technician responsible for its collection or analysis.

DNA technology has advanced greatly since its inception, but the techniques for collecting and handling evidence have not matched the pace. From the crime scene to the autopsy table, protocols for preventing DNA contamination are necessary to avoid blemishing an exhibit and impeding an investigation (Schwark, 2011). Implementing strict evidence handling standards will reduce DNA contamination and its consequences.

The Power of the Polymerase Chain Reaction (PCR)

Every effective forensic laboratory utilizes PCR to amplify minute amounts of DNA to derive an identifiable profile. PCR amplifies short tandem repeats (STRs) within a DNA sequence and electrophoresis then separates the STRs by length, and visualizes them as peaks on an electropherogram (Gilbert, 2010). Each individual (excluding identical twins) possesses a unique set of STR peaks that creates an identifiable profile. The standard analysis of a DNA sample requires around 200 picograms of DNA, or roughly 33 cells of DNA material (Gilbert, 2010). New methods will amplify DNA that cannot even be visualized; for example, low-copy-number analysis, which has the ability to generate at least partial profiles from just a few human cells.

Low-copy-number (LCN) analysis does have some downsides despite its impressive sensitivity levels. A ‘drop-out’ or ‘drop-in’ effect heavily distorts any type of effective analysis (Gilbert, 2010). STRs present in the original sample may ‘drop-out’ and fail to appear in subsequent visualization, while contaminants in the PCR may cause STRs to ‘drop-in’ to the results (Gilbert, 2010). Either phenomenon poses a risk to the integrity of the sample, as it alters the profile readout and may mislead the technician analyzing or comparing it.. A technician performing PCR analyses should strive for an accurate and contaminant-free amplification process rather than a semi-accurate hyper-amplification of samples likely to contain some form of contamination or distortion (Gilbert, 2010).

The Amanda Knox Murder Trials

Contamination has the potential to affect a criminal investigation long after the crime occurs; a case introduced to the judicial system with contaminated evidence will in turn have a

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contaminated verdict. The Amanda Knox murder trials were held within the Italian justice system, but the contamination issues are nonetheless relevant to American systems and standards. Amanda Knox was suspected of murdering her roommate, Meredith Kercher, in 2007, despite DNA evidence linking another individual to the scene. Investigators ignored blood and fingerprint evidence to instead focus on several of Kercher's cells found on the blade of a kitchen knife found in the apartment shared by the women (Hogenboom, 2014). Knox's DNA was on the handle of the knife, and the identification of Kercher's DNA on the blade was enough for a conviction. Amanda Knox is now facing a third re-trial after her first two guilty-verdict trials were appealed and overturned. Experts are now arguing that Kercher's DNA was detected on the blade as a result of contamination during the evidence handling process (Hogenboom, 2014). If this was a true contamination event, then an enormous amount of Italy's resources have been spent fighting appeals and investigating Knox based on unsound forensic evidence. Over six years have passed since Kercher's murder. Claims of poor crime scene containment have created further controversy in the case, as sources noted multiple people entering and leaving the room where the murder occurred, and investigators without protective clothing (Hogeboom, 2014). Knox's defense further argue that appropriate laboratory procedures were not followed and certain evidence items were handed back and forth between investigators, possibly initiating contamination events (Hogenboom, 2014). These issues have drawn out the timeline, and still there hasn't been any closure or justice for those involved. If appropriate protocols were in place before this incident, fewer questions would remain about what actually took place that night in 2007. The Knox trials show the potential that

contamination events have to wreak havoc in the judicial system; even a few cells can be the deciding factor in a conviction.

Removing Unwanted DNA

There is no one-step method to remove contaminant DNA from a sample. A variety of sterilization techniques exist that utilize ionizing radiation or other chemical treatments (Shaw et al., 2008). Some of these methods are also hazardous and require extensive precautions and training to perform them.

Comparison of Four Sterilization Methods

Shaw et al. (2008) compared the effectiveness of UV, gamma, and electron beam radiation as well as the reagent ethylene oxide to remove unwanted DNA contaminants. Using varying amounts of saliva on both porous and nonporous surfaces, all four methods were performed in triplicate and amplified with polymerase chain reaction (PCR). Sterilization with UV radiation did not degrade sufficient DNA from the sample; 100% of contaminated samples provided full DNA profiles following this technique. Gamma and electron beam sterilization was most effective with small volumes of DNA (1-2 μ L) but was not as effective with larger amounts. Only the electron beam radiation had the ability to remove all DNA contaminants with a 3% success rate. Ethylene oxide proved to be the most efficient of the researched techniques, with 13% of samples producing no DNA profiles upon analysis. No difference was found in the effect of surface (non-porous or porous) on subsequent sterilization and recovery of DNA profiles during this experiment. Researchers concluded conventional techniques used for sterilization do not guarantee complete, consistent removal of contaminant DNA. Ethylene glycol was found to be most effective of the tested techniques for DNA removal, and is recommended by the authors for

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sterilization of laboratory equipment made of plastic or metal. The use of ethylene glycol is restricted to smaller items and is not a common method readily available to the average crime lab, but has the most promising results for removing any amplifiable DNA within a sample.

Further Study into UV Irradiation and Reagent-based Decontamination

The UV radiation technique was further studied and compared to chemically based decontamination methods in 2009 (Preusse-Prange et al., 2009). Researchers found an increase in decontamination as distance lessened between the UV source and the sample, while exposure time (ranging from 5 minutes to 24 hours) had no effect. It was also determined that a shorter wavelength of UV light was able to reduce the presence of DNA in a sample with greater efficiency. Despite these results the authors of the study only attribute the tested methods to contamination reduction as opposed to complete elimination. The importance of avoiding contamination prior to any laboratory analysis is a vital issue as long as current technology is unable to fully eliminate contaminants.

Removing PCR-Related Contaminants

Even if a few DNA molecules from a previous examination contaminate a PCR reaction, the amplification possibilities of the technology pose a risk for future analysis. After a selected fragment of DNA is amplified, it will have dUTPs in it; something unamplified DNA does not possess. Crime labs couple PCR with an enzyme called uracil-N-glycosylase (UNG), which degrades any unwanted amplification products from the sample (Pruvost, Grange & Geigl, 2005). UNG can be activated and inactivated as the PCR reaction is performed to ensure any unwanted DNA fragments from

previous analyses do not contaminate subsequent cycling. UNG-coupled PCR is an effective technique to reduce risk of contamination during the amplification process, and should be utilized as a preventative measure whenever possible in the forensic laboratory. Although UNG-coupled PCR removes contaminants related to PCR processing, it cannot degrade a contaminant that was present in the sample before the analysis was performed.

Sources of DNA Contamination

DNA Transfer Upon Manufacture

Manufacturers of DNA instruments and equipment must also take great care to avoid DNA contamination during the manufacturing process. Some major companies like Promega have taken steps to minimize the occurrence of contamination by including recommendations for elimination databases, automated contamination checks, and national logs for contamination events. If companies that manufacture the collection swabs or evidence bags required for sterile crime scene collections do not take certain measures to prevent contamination, extensive police resources could be wasted on possibly flawed forensic results.

The Phantom of Heilbronn

Known as “The Woman Without a Face”, the Phantom of Heilbronn was one of Germany’s most-wanted women, leaving DNA evidence at 40 crime scenes, including various burglaries and six murders, across Europe between 1993 and 2009 (Spiegel, 2009). Countless resources were spent trying to locate the Phantom and bring her to justice, particularly after her DNA was found during the investigation of a police officer’s homicide in Germany. It took over a decade for investigators to determine the true identity of the elusive Phantom. In 2008, French police swabbed the body of a burnt male to attempt a DNA

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identification of his body, and were surprised to find the DNA of the Phantom as well (Spiegel, 2009). After inquiry into the equipment being used to process the Phantom scenes, it was discovered the Phantom was in fact a Bavarian woman working in the factory that manufactured swabs for investigative use. Thousands of hours had been spent investigating a woman who had no involvement in any of the crimes, all due to contamination. The extensive investigation into the identity of the Phantom could have been avoided if standards similar to those suggested by Promega were implemented. A forensic laboratory with access to employee DNA databases and required contamination checks would have identified the donor in weeks rather than decades.

DNA Transfer at the Crime Scene

Fingerprint Brushes and Powder

Sources for DNA deposits at a crime scene can include any item that has come into contact with an individual or their bodily fluids (saliva, sweat, semen, etc.) (Blozis, 2010). Latent fingerprints often contain enough skin or sweat to provide a full DNA profile of the donor. Therefore, great attention must be given to the fingerprint brushes and powder used to lift these latent prints from a scene in order to avoid DNA contamination and cross-contamination. Nonetheless, it is common practice to use the same brush to powder different objects at different scenes (van Oorschot, Treadwell, Beaurepaire, Holding & Mitchell, 2005).

Squirrel-hair brushes are frequently used in the United States to process latent fingerprints with black powder. A 2005 study conducted by Van Oorschot et al. tested the potential for DNA transfer with used brushes and powder. Some of the brushes used were used in casework, but others were purposely contaminated

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with handprints, saliva, blood, or a mixture of the three. After analysis, both full and partial DNA profiles were recovered from used brushes and used powders, and transfer was occasionally seen between brushed surfaces (van Oorschot et al., 2005). Transfer was also noted between several subsequently brushed sheets of plastic following contact with dried saliva stains as well. Van Oorschot et al. (2005) advise all powder should be removed from the sample before amplification is performed, as powder presence seems to inhibit the PCR process. Overall, fingerprint brushes were shown to accumulate DNA and redeposit it to subsequently brushed items; this problem will worsen as DNA typing methods become more sensitive. Van Oorschot et al. (2005) provided recommendations to prevent contamination through fingerprint brushes or powder:

- Use alternative techniques to develop fingerprints without making contact with the print.
- Use separate, disposable brushes for powdering each object to avoid transfer.
- Prepare and use separate aliquots of powder so the same container is not being used for long periods of time.
- Avoid all contact with biological samples when possible.
- Develop more extensive sterilization methods for fingerprint brushes.
- Avoid applying powder to areas that you believe may be swabbed later for DNA collection.
- Pay attention to the type and condition of surface being brushed.

Glass fiber and bird feather fingerprint brushes are used more commonly in European countries. There, like American jurisdictions, brushes are typically used by departments for

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several weeks and up to months at different crime scenes (Proff, Schmitt, Schneider, Foerster & Rothschild, 2005). Secondary transfer of DNA was also seen with both used and purposely contaminated brushes of this type, and research found that certain individuals could be considered “good DNA shedders” (2005, p. 602). The larger the area being powdered, the greater the likelihood of DNA transfer. Contamination while using these types of brushes was avoided if brushes were changed out between important exhibits or crime scenes. Proff et al. (2005) also suggested development of decontamination procedures for glass fiber and bird feather brushes, as current protocols are insufficient to prevent contamination.

Evidence Packaging and Transport

Departments have a large variety of evidence packaging materials to choose from. Each manufacturer provides standards for handling their products, but it is ultimately up to the department to develop a collection protocol for exhibits (Goray et al., 2012). In some cases, when an exhibit reaches the laboratory for analysis, the DNA has been “lost” or has been transferred to other areas of the evidence or its packaging due to improper evidence containment choices or other technician ignorance (2012). Goray et al. (2012) researched the potential for DNA transfer within evidence packaging through multiple trials with various packaging and scenarios (multiple exhibits in one bag, paper or plastic containment, etc.) and concluded that transfer is a likely occurrence (2012). Several tested scenarios showed issues with loose packaging, deposits on cotton material, and the collection of multiple exhibits in one bag. The strongest recommendation of Goray et al. (2012) was to package all evidence items separately. The evidence technician cannot be sure about the source of DNA at collection; therefore, all

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evidence should be packaged individually and supplemented with observations at the time of retrieval to prevent later confusion during analysis.

DNA Transfer at the Forensic Laboratory

Even if the packaged evidence arrives at the forensic laboratory without contamination, preventive measures must continue to ensure no foreign DNA is deposited during analysis.

Exhibit Examination

Physical examination of an evidence item requires contact between the technician's tools and the exhibit. According to the aforementioned Locard's Exchange Principle, trace evidence of that contact will exist. In one study, mock forensic casework was performed to simulate examination with forceps, scissors, and gloves and varying contact times. Szkuta, Harvey, Ballantyne & van Oorschot (2013) found that DNA was transferred for all tools in all scenarios, the only exception being the forceps under brief-contact conditions. The tools analyzed were shown to have a greater contamination risk if used incorrectly; contact between tools and exhibit areas to be tested for DNA should be avoided if possible. If this cannot be accomplished, all tools that come in contact with suspected DNA samples must be sterilized or replaced immediately (Szkuta et al., 2013). The high potential for DNA transfer seen with forceps, scissors, and gloves highlights the necessity for heightened awareness of contamination even in a "sterile" laboratory.

Another study performed by Finnebraaten, Graner, and Hoff-Olsen (2008) examined the hypothesis that a speaking individual could contaminate an exhibit he or she is sitting or standing over. Subjects dressed in full protective equipment without a facemask repeated a sentence for 5 minutes and 1 minute in both the standing and sitting position. Full DNA

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profiles were derived from the standing test group, but partial profiles were more prevalent (Finnebraaten et al., 2008). The presence of even a partial profile from speaking individuals in a workspace raises concerns about the same kind of contamination of evidence occurring at the crime scene. Extra care must be taken when handling evidence without appropriate protective equipment; even speaking has the potential to compromise the subsequent interpretation of evidence items.

DNA Transfer in the Superglue Chamber

A superglue chamber is used in the forensic laboratory to develop latent fingerprints for easier visualization and analysis. Superglue is heated and turned to vapor in a controlled chamber with the evidence item. The superglue will bind to latent prints present on the object and make them visible. Because the chamber contains vaporized particles, movement of particulates within it is very possible. Gibb, Gutowski & van Oorschot (2012) swabbed a superglue chamber that had been in use without cleaning for several years. The chamber was then cleaned and tested again after certain numbers of fumigations had been performed. It was shown in this preliminary research that DNA has the potential to accumulate and transfer within the chamber. Gibb et al. (2012) suggested new standards to prevent DNA buildup and subsequent transfer:

- Incorporate filters or UV lights into chambers to degrade DNA between fumigations.
- Clean the chamber with appropriate reagents between fumigations.
- Place blotting paper at the bottom of the chamber that is changed out with each new fumigation.

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- Maintain a staff DNA database to quickly rule out foreign contaminants, and a log for all employees to fill out when the chamber is used.
- Regularly take swabs from the chamber and analyze them to ensure no DNA is accumulating.

DNA Transfer During Autopsy

Contamination can occur as a body is transported to the morgue as well as during autopsy (Schwark, Poetsch, Preusse-Prange, Kamphausen & von Wurmb-Schwark, 2011). Schwark et al. (2011) investigated what kind of DNA transfer was possible within an autopsy environment. Common tools used for each autopsy were tested for the presence of DNA profiles after sterilization; these items included measuring sticks, tables, neck rests, and forceps. A high contamination risk during forensic post-mortem examinations was found to exist and transfer between the table and the body being autopsied (Schwark et al., 2011). The only sanitation solution that fully removed DNA contaminants from autopsy tables was commercial bleach cleaner). Schwark et al. (2011) recommend monitoring autopsy tables for DNA material in between examinations to ensure that contaminants are not transferred over, or collecting DNA profiles from the deceased to have a database to refer back to in case contamination occurs.

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Table 1
Recommendations to Reduce Contamination in Forensic Science

Location of Contamination	Contaminated Object(s)	Contaminant Prevention
Manufacturer	Equipment used for sterile crime scene collections	<ul style="list-style-type: none"> -Maintain DNA databases with employee profiles -Perform quality checks to ensure no contamination in final product.
Crime Scene	Fingerprint brushes and powder	<ul style="list-style-type: none"> - Decrease contact between latent print and developer; any brush that touches suspected biological material must be replaced - Use disposable or sterilized fingerprint brushes -Prepare small powder aliquots
	Evidence packaging	<ul style="list-style-type: none"> - Individually package <u>all</u> evidence collected from a scene - Avoid use of loose packaging
Forensic Laboratory	Forceps, scissors, gloves	<ul style="list-style-type: none"> - Sterilize / replace all items that come into contact with biological samples - Increase awareness of contamination risk when handling evidence
	Superglue chamber	<ul style="list-style-type: none"> - Use filters/UV rays to degrade DNA between analyses - Clean chamber with appropriate reagents - Regularly test the interior of chamber for contaminants
Morgue	Autopsy table and related equipment	<ul style="list-style-type: none"> - Sterilize surfaces and objects used for multiple cases with commercial bleach cleaner - Create DNA database for deceased individuals processed at the facility

Conclusion

Research compiled worldwide over the past decade has focused on areas of forensic science vulnerable to DNA contamination. Researchers have identified a lack of standards and appropriate protocols as the primary problem. Table 1 shows known contamination sources and the research-recommended remedies for associated contamination events. If not addressed, DNA contamination will continue to beget financial and social costs, including potential convictions of innocent people. As the DNA technology advances in sensitivity, greater consideration must be given to the possibility of contamination, and its resultant consequences, by adopting firmer protocols regarding DNA evidence.

Further research devoted to the prevention of contamination should investigate a more universal reagent to remove all DNA from forensic collection and analysis equipment before use. A controlled method to remove DNA contaminants that can be utilized as needed – from the crime scene to the morgue – will decrease the overall prevalence of contamination events in forensic science. Contaminant DNA research is also needed in the area of crime scene equipment used in evidence collection, such as swabs and evidence bags. The development of sterile, recyclable equipment would be of great use to financially-strained departments.

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