ENSURING THE INTEGRITY OF RESULTS: A CONTINUING CHALLENGE IN FORENSIC DNA ANALYSIS

Trevor Howitt
Forensic Science Service

Introduction

In recent years, the sensitivity of DNA analysis techniques has increased to such an extent that we can now obtain profiles from as little as a few cells and from contact traces that are invisible. As a result, the risk has also increased of detecting extraneous DNA, unrelated to the crime itself, in samples taken at the crime scene. The extraneous DNA may have been present for entirely innocent reasons at the crime scene prior to the crime being committed, or it may have been deposited inadvertently after the incident. Potential sources of such post-incident inadvertent contamination include police investigators attending the scene, scene of crime officers collecting samples for laboratory examination, the laboratory staff who process the samples and contaminated materials used in the processing of the samples.

A raft of measures have been introduced over the years to reduce the level of such post-incident contamination, and to improve our ability to detect it and take due account of it when it does occur. For example, we now have access to elimination databases containing DNA profiles of FSS staff and police personnel to allow checks to be made to determine whether the profile obtained could have originated from individuals closely involved in recovery and processing of the sample.
We also have quality control measures in place to check for systematic contamination of the consumables and reagents we use in the DNA analysis process, and to check for contamination between samples.

Despite these extensive precautions a risk remains of contamination occurring and not being identified as such. This paper describes a recent investigation into unusual links observed between cases in the UK which has led the FSS to re-appraise our own anti-contamination measures and those required to ensure the integrity of data held on the National DNA Database (NDNADB).

An Investigation into Unusual Casework Links

Casework Example A

During the high profile investigation of a murder of a young girl, items of the victim's clothing that were unconnected with the scene of crime were subjected to DNA profiling and one of these items yielded a full male profile. The NDNADB was used to provide intelligence information that suggested links between this male profile and two other unsolved crimes, a burglary and a drugs-related offence in different parts of the country.

Casework Example B

In March 2002 the burnt body of a female murder victim was discovered in a London public park. A toothbrush from the suspected victim's house was submitted to the laboratory for DNA analysis as a means of helping to confirm her identity. This yielded a mixed DNA profile comprising the victim's DNA profile and a minor component of male origin. The male profile did not match any individual connected to the case, but separate DNA evidence recovered from the crime scene did match one of the key suspects. Later in the investigation a speculative search of the National DNA Database was carried out on the male profile from the toothbrush to see if any person from whom it might have originated could be identified. This identified matches with crime scene DNA profiles from 4 unsolved burglaries and thefts committed in a defined area of northern England hundreds of miles from the murder scene. It should be noted that this linking profile was different to that observed in Example A.

The links to minor offences observed in both of these entirely separate murder investigations were considered to be unexpected and unlikely by the respective investigation teams, given in both cases the differences in location and offence type. Contamination was a plausible explanation; hence an internal inquiry was instigated by the FSS to try to determine the cause. Comparison of the linking profiles against the staff and police elimination databases failed to provide any matches. However,

matches were identified when comparisons were made against records of contamination seen in negative controls for batches unrelated to the casework samples in question. For Case Example A, 2 partial profiles matched from negative controls run with batches in June '02 and October '02. Re-amplification of one of these negative control samples under LCN conditions (i.e. 34 PCR cycles compared with the standard 28 for SGM+) gave a more complete profile with a random match probability of less than 1 in a billion. For Case Example B, nine profiles matched from negative controls run between February 2000 and February 2003. Further checks revealed that the profile had also been observed during QC checking by the FSS of a batch of microfuge tubes that had not been accepted from the manufacturer due to the presence of the contaminating profile. The most plausible explanation therefore was that DNA contaminants were being introduced sporadically into the consumables used in DNA testing, most probably the microfuge tubes.

Negative Controls Detect both Systematic and Sporadic Contamination
The investigations detailed above led to the realisation that the negative control log information could be used in a new way to detect potential contamination sources. Previously, the negative control samples had been routinely used only as a check that the batch was free from systematic contamination. Data was thus held locally by each of 6 laboratories retaining details of any full, or more typically partial, profiles observed in the extraction and PCR negatives which remained unsourced, i.e. could not be attributed to a member of staff or a sample processed in the same batch. However, by combining the negative control data from all laboratories in a single log, valuable information can de derived regarding contamination that is from a single source but occurs sporadically over time as tube specific events. Searching this collated data against the NDNADB identified, in addition to the 2 profiles highlighted in Examples A and B, a further 9 different profiles in the negative controls which were also found to match full or partial profiles, or components of mixed profiles, attributed to crime scene samples. Again it was reasonable to assume that these profiles in the negative controls also originated from consumables used in the DNA process. To put this level of contamination into context, these were observed over a three-year recording period, during which time over a million samples had been processed through the laboratories.

Identification of the Contamination source.
The tube manufacturer, whose production is outside the UK, was approached regarding supply of staff samples for elimination purposes, and responded positively by providing, on an anonymised basis, over 300 samples from their factory floor staff. These have yielded full matches to 10 out of the 11 identified casework-contaminating DNA profiles, including both those observed in Casework Examples A and B. These results clearly demonstrate that the microfuge tubes used in DNA processing were the source of a number of different contaminating DNA profiles.

Lessons Learned: Improved Anti-Contamination Measures

In response to the issues raised from this investigation, and to the continuing challenges of improving data integrity, we are addressing DNA contamination from all perspectives by introducing measures to minimise its occurrence, maximise our ability to detect it and to take it into account in the assessment of casework:

Reduction in Sporadic Contamination of Consumables
Feedback was given to the microfuge tube manufacturer throughout this investigation. Additional anti-contamination measures were introduced into the production process, and in recent months we have observed a very marked reduction in the number of contamination events attributable to their manufacturing staff.

In general terms, improved communication is required with some suppliers of consumables regarding DNA contamination issues if the needs of the forensic community are to be properly met. For instance in the past we have encountered potential suppliers who have failed to realise that guaranteeing sterility is not the same as providing a DNA-free product. With another company general lack of forensic awareness had resulted in production staff being equipped with gloves and hairnets but not facemasks because the risk of contamination from saliva aerosols had not been recognised. Worryingly some forensic laboratories just assume that these consumables are DNA-free and accept materials without any form of QC testing.

It should also be emphasised that critical consumables used at each stage in the collection and processing of samples need to be considered, and not just those used within the testing laboratory. It is essential therefore that the kits used by the police at scenes of crime to recover DNA evidence and those utilised for the collection of reference samples from suspects and victims are also manufactured in DNA-free conditions and subjected to appropriate QC-testing.

It is impossible to carry out QC checks on individual items of consumables, such as tubes, tips and other components, to guarantee that they are DNA-free prior to use, because once they have been QC tested they are discarded. However, QC testing of a sample of components from a batch can help provide some indication as to the general batch quality. Even so, it will only show whether gross systematic contamination is present and it cannot provide a guarantee that sporadic contamination events have not occurred.

Even when manufacturing has been undertaken under conditions to prevent DNA contamination as far as is practicable, additional precautions should be taken to counter sporadic, and therefore virtually undetectable, contamination events. Critical consumables should be exposed to physical or chemical treatments to ensure that any DNA that may be present is destroyed. Inclusion of controls within the batch enables the efficacy of treatment to be verified and provide an additional level of confidence that the consumables are free of DNA contaminants.

Increased Process Automation.
Direct comparison of manual and automated sample processing lines within the FSS for both database reference samples and some casework material has consistently demonstrated that automation of processes reduces the risk of contamination occurring. Aside from effectively eliminating human error from most of the process, automation also reduces the potential for staff to contaminate with their own DNA, and offers other significant benefits including more consistent quality of results and significantly reduced costs per sample processed. Given these benefits we are in the process of widening the application of robotics to the processing of a greater range of evidence types.

All casework DNA profiles for which a source is not attributable are now routinely screened by the FSS against the Staff Elimination Database and the contamination log. The contamination log comprises profiles from batch extraction and PCR negatives controls, profiles occurring in QC testing of consumables, and results from routine environmental monitoring of the laboratories. These checks are undertaken prior to the results being reported as evidence or added to the NDNADB.

A Police Elimination Database has also been established of some 70,000 scene-going staff, against which checks can be made. However these checks are presently limited to the comparison of recovered DNA against specifically named staff pertinent to a specific case under consideration, and are not as yet undertaken on a routine basis.

A National Contamination Log
A national contamination log is currently being established and will be made available to all suppliers of DNA profiles to the UK National DNA Database. Initially this will be populated with the unsourced contamination profiles held by the FSS but it is expected that all suppliers to the NDNADB will contribute on a non-competitive basis. This will increase our collective forensic ability to detect sources of sporadic contamination, especially where more than one supplier to the Database share a common source of consumables. For example, prior to this investigation, one of the 11 aforementioned contaminating DNA profiles had already been separately observed and reported to the police in 2 unrelated cases, one being dealt with by the FSS and the other by a different supplier to the NDNADB who utilised the same make and type of microfuge tubes. Thus this contamination issue is not specific to our organisation. It is potentially a global issue for all forensic DNA laboratories.

Expansion of Elimination Databases
Although checks against staff elimination databases are common practice in many laboratories, it is evident that this philosophy of sampling laboratory staff most at risk of depositing extraneous DNA and routinely screening against their profiles for elimination purposes now needs to be expanded. The following should also be included in the future: all individuals attending scenes of crime; any individuals such as cleaners, maintenance engineers from equipment suppliers, and defence experts

who require access to sensitive areas within the laboratory; other staff working within the laboratory but not in sensitive areas, given the risk of DNA contamination occurring through secondary and tertiary transfer; and the production staff of manufacturers of key consumables used in the DNA analysis process.

The response of the microfuge tube manufacturer in the aforementioned incident was exemplary, given that they voluntarily provided samples, and we are actively seeking to do the same with all other suppliers of critical consumables. In the future the forensic community should be insisting that a condition of contract for supply of these materials is that a manufacturer's elimination database is established.

Automated Checks for Potential Contamination.
Introduction of a fully integrated approach to DNA profiling has entailed development of robotics for extraction and amplification of samples in a microtitre plate format, followed by processing the PCR product by means of automated CE instruments,combined with automated data processing by development of expert systems. This approach has provided an ideal platform to develop in-house a number of expert systems designed to detect and deal with potential contamination. For instance the spatial arrangement of samples is now constant in an 8 x 12 grid throughout the process, enabling simple automated checks to be run for potential sample to sample contamination events. These systems present a significant step forward in speed, reliability and quality of processing, allowing the FSS to factor in the following checks:

- Assessments using peak area to look for low-level or degraded components.
- Automated searches against staff profile lists and other known contaminants.
- Use of case information, proximity of samples and closeness of matches to sift and prioritise checks.
- Provision of real-time management information on the contamination rate and therefore cleanliness of the laboratory.
- To statistically correct for potential contamination when reporting evidence in the Court.