

TRACE EVIDENCE – SMALL SAMPLES, BIG PROBLEMS

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ABSTRACT: This lecture will look at the scope of forensic trace evidence and will identify methods for analysis. Precautions to be taken as a part of a programme of contamination avoidance will be highlighted and the requirement for databases and background data will be discussed as a requirement for the interpretation of all types of trace evidence.

KEY WORDS: Trace analysis; Contamination; Evidence interpretation.

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INTRODUCTION

Before we can discuss the problems associated with trace evidence it becomes necessary firstly to define what is meant by the term “trace evidence”. Traditionally we think of trace evidence in terms of particulate matter particularly samples such as paint, glass or building materials that are, with experience, easily observed with the aided eye, removed from their individual matrices and analysed. The recovery of fibres and their analyses also falls into this class of trace materials. Other, less traditional materials which are usually or can be referred to as “trace evidence”, include biological fluids, explosives, fire and firearms discharge residues. The first of this latter group is distinct in that its presence, usually on clothing, can range from gross quantities to trace levels. While the position of particulates on clothing does not always indicate the original position of contact under Locard’s exchange rule because of activities leading to their redistribution, the same cannot usually be said of body fluids where the position of contact is plain to see and where the distribution can be extremely important in the interpretation of how such samples arose. There may, however, be a third type of trace evidence and that is where minute changes in the character of surfaces become important. This is observational trace evidence such as may be found by the distortion of surfaces caused by the defects in a cutting implement or characteristic distortions found on the cartridge of a discharged round of ammunition, but not something that can be physically placed inside a container. These

latter are often associated with reconstruction exercises designed to unravel a series of events associated with a crime.

Let us now take a step backwards because what we have defined is “traces” while what is the topic of this communication is “trace evidence”. The question we have to ask is when do traces become “trace evidence”? This phrase has both a scientific and a legal meaning; in the former it represents physical items that have been recovered or observed by the forensic scientist during his/her scientific investigation of a scene(s), or items, often recovered from clothing, but thought to be associated with a crime. Some, but not necessarily all, may be analysed in order to produce information that will link or suggest a link between an item and an additional item or a link between a person and an item or a link between two persons or a link between two or more sites. The forensic scientist is only in a position to identify what is likely to be of evidential importance from information adduced as a crime scene investigator or from information received from an investigating officer. This latter makes it vitally important that information is accurately and comprehensively passed from the investigator to the scientist.

There is another aspect of trace analysis and that is its use as a proactive investigative tool where the finding of a trace material may open up a line of investigation. That is to say trace evidence becomes an intelligence source. Examples would be the finding of traces of explosives on a premises as an indicator that the premises may have been used to construct an explosive device or DNA profiles recovered from the insides of drug packages. Clearly some of this information would only be of use if associated with a relevant data base.

In the legal context such physical traces only become evidence once they are formally submitted to a court in the form of exhibits or productions.

It is important to realise that the quantities involved in trace analysis work covers a range of mass values from 10^{-3} to 10^{-12} g and for this reason a number of factors must be taken into consideration.

RECOVERY OF TRACE MATERIALS

Generally there are well established procedures for the recovery of trace items from different matrices. For example the recovery of tiny glass or paint fragments can be accomplished by pure physical means with the aid of a search microscope, while fibres are usually recovered using taping procedures in which transparent adhesive tape is pressed onto a surface and then onto some transparent medium for microscopical searching for relevant fibres. Adhesive surfaces may also be employed for the recovery of inorganic firearms discharge residues. Other means of recovering such items are

sometimes employed which include scraping and shaking of garments. In those cases where very small particulates are being sought such as would be true for fine building materials or traces of explosive powders then recourse may be made to vacuuming systems. Where no particulate material is under examination, trace material may be recovered by solvent extraction. This would be true for the recovery of body fluid samples which would employ aqueous salt or buffers solutions and trace explosives which might employ alcohol as a solvent. Before such methods are chosen a number of factors must be taken into account:

- Will the procedure recover the maximum amount of trace material present?
- Will the recovered material using the proscribed method be free of contamination arising from the matrix in which the trace sample originally existed?
- Can it be established that the recovery materials themselves do not change the nature of the trace sample?
- Are the materials used to recover trace samples free from contamination?
- Is the environment in which the recovery process is undertaken free from contamination?
- Has the identical sampling procedure been used on control samples at all stages of the isolation procedure and at the same time?

It is axiomatic that all examinations for trace materials must ensure that all proper controls have been taken and that these controls are sufficient and representative of the item under consideration and are themselves free from contamination. Associated with this is the question of packaging which, for trace evidence, must be demonstrably secure and to support this control package materials become important. For example, it is not acceptable to use polythene bags to store trace explosive or fire residue samples because of their permeability to such samples but it would be expected that an empty nylon bag of the same type and batch used to store fire residue samples would be taken as a control. Additionally, common sense would dictate that it would be invidious to use a glass vessel to store tiny fragments of recovered glass! In respect to this latter sample before any analyses can be conducted the surface of the glass must be cleaned usually with nitric acid, water and finally acetone.

These procedures may still produce a product that is unsuitable for analysis especially when using the more sophisticated instrumentation whose system is easily contaminated. A typical example would be a mass spectrometer used to analyse for explosive traces. The response to this problem is the use of a preliminary clean-up procedure. Such procedures must meet the criteria of efficiency, that is, there must be little loss of the trace sample when

using such procedures but they should also be rapid to cope with the increasing workload experienced by all forensic science laboratories. Additionally, such procedures must be specific for the particular analyte and demonstrably free from contamination. This latter is usually achieved by running a parallel experiment of controls at the same time. Examples of preliminary clean-up procedures would be for the isolation of explosive traces and in purifying DNA samples, both of which make use of an appropriate synthetic resin.

For some trace materials that are complex and distributed over large areas or volumes there may be a requirement to concentrate such materials in order for them to be accessible in an analytically useable form. Typically the matrix employed consists of a selective strongly adsorbing surface such as Tenax or activated charcoal. The analyte may then be desorbed from such matrices either by thermal means such as is employed in the Perkin-Elmer ATD instrumentation or by means of solvent elution with alkanes or carbon disulphide. Such systems are employed extensively for the recovery of accelerants from fire residues but can be employed for all types of volatile and toxic materials.

While these types of preliminary clean-up methods are adequate for many types of trace evidence there are some types of such evidence that are not able to comply with these procedures but for operational needs there is a requirement to speed up the isolation of a targeted analyte. Perhaps the best example of this is the isolation of target fibres from a forest of background fibres found on a tape lift taken from a garment. The tedium of isolating the target fibre from such a matrix has been long recognised so that the introduction of fibre scanners in the last few years has been received with approval although they still have some limitations.

ANALYSIS OF TRACE MATERIALS

The analysis of trace materials may often go through a two-stage process, the first stage being a screening test and the second one or more specific tests. It is here that there may be some conflicts in philosophy especially in the first stage. Because we are dealing with trace materials it is important that any screening technique does not use up the limited sample available. For some trace materials this has led to the use of sophisticated instrumentation in the screening process. A typical example would be the use of EGIS and the IONSCAN mass spectrometry. These instruments show a high degree of specificity for explosive traces but in the United Kingdom are not considered specific enough in themselves for an identification that can be brought before the courts. The question then arises would a response from

both such instruments be sufficient? Some believe this to be the case others believe that this is not acceptable. These views are further coloured when the instruments are used either at the scene of an incident where contamination problems could arise or under strict laboratory control. This debate remains an open one.

Analyses for trace materials have come a long way in recent years and are highly dependent upon sophisticated instrumentation where the aim is almost always to uniquely relate one sample or item to another. With all this sophistication one must never forget that it is the answer to a problem that we are seeking and not an analytical exercise and the solution to the problem may not require the high sophistication we may think we need. For example, the simple measurement of a refractive index for a glass fragment recovered from clothing may eliminate the fragment as of coming from a particular source without the necessity of an ICP/MS analysis. Glass also possesses other properties that may enable one fragment to be distinguished from another. For example, characteristic surface scratches and pitting, any change of refractive index on annealing, the presence of multiple refractive indices on a single fragment and the presence of a flat surface. This will mean that we have important analytical choices to make before embarking upon an investigation and these choices will be based upon what procedure is likely to answer the question that is being posed in the fastest time with the greatest surety. There will also be other decisions to be made when for example there are mixed trace samples such as lubricants and body fluids. Under these circumstances any decision must take into account the likely evidential value attached to the successful analyses of each of the components and whether the processes of recovery of the separate trace evidence types will destroy one of the components of the mixture? Such decisions often apply to samples other than those of a trace nature.

The quality control in the manufacture of most materials in today's world means that there is less variation in the composition of materials than once was the case. This has meant that the forensic scientist is looking for those small differences that may still be present but which may be much harder to find. In this context it is therefore important to be able to demonstrate not only differences between batches but also differences within batches of the same product. An interesting example of this was a survey of the blue dyes present on fibres taken from a collection of socks and also recovered from the uniforms worn by police officers [10]. It became clear here that by a simple TLC analysis of the dyes, companies use several different dye mixtures to produce the same colour and that these mixtures may change from batch to batch. One company used as many as 20 different dye compositions to meet the colour requirement. This means batch variation can of itself be of importance for the analysis of mass produced articles but within batch variation

was much more difficult to detect. In the area of glass analysis progress has been made beyond batch discrimination when using trace metal analysis in that samples can be allocated to specific glass manufacturers and their sources of raw materials (see [1] and others). Further progress in this latter area is dependent upon the generation of extensive databases but the complete individualisation of glass fragments is still unattainable and perhaps never will be by chemical means?.

What therefore are the methods being employed for the analysis of trace materials? A list of currently used procedures is given in Table I.

It is clear from such a list as is give in Table I that there are a number of key instruments that are required for the analysis of trace materials, these include comparison microscopy some form of mass spectrometry, scanning electron microscopy, gas and high performance liquid chromatography and, infrared microscopy. It is difficult to imagine a modern forensic science laboratory which does not possess such equipment but their use can generate problems if the proper precautions are not in place. For example it is easy to fall into the trap of the “Black Box” syndrome in which the operational principles of the instrument operate under a mystical presence to produce a “printout” which must be correct because the instrument says it is so. Such equipment still requires an input from an operator and a knowledge of the principles of operation sufficient to identify that the instrument is working to specification and is not in anyway contaminated. This of course requires good laboratory practice in the running of blanks or negative standards, and positive standards as well as controls generated along with the sample to be tested. Where quantitative results are a requirement then the linearity of response must be continually monitored by the use of standards of known concentration. All this presupposes that the analytical process has previously been validated and that the precision, accuracy, linearity of response, sensitivity and specificity is known. A useful check list therefore would be:

- How does the analyst know that the equipment is working to specification at the time of the analysis?
- How specific is the test for an individual evidence type and what is the analytical “window” permitted for a positive identification?
- Is the technique sensitive enough to detect the proscribed level of that trace evidence type?
- Where quantification of the evidence type is required, what is the accuracy and precision of the analytical technique both within days and between days?

TABLE I. LIST OF TRACE EVIDENCE TYPES AND ANALYTICAL PROCEDURES USED FOR THEIR CHARACTERISATION

Trace type	Methodology
Body fluids	DNA using the polymerase chain reaction
Building materials	Scanning electron microscopy (SEM), X-ray diffraction (XRD)
Fibres	Comparison/fluorescent microscopy, microspectrophotometry, Fourier transfer infra-red microscopy (FT-IR), dye analysis (TLC, HPLC with diode array detection, DAD)
Fires/accelerants	Gas chromatography (GC) with mass spectrometry (MS)
Glass	Refractive index, SEM and variations on inductively coupled plasma emission spectroscopy (ICP) including inorganic MS
Gunshot residues (sometimes referred to as cartridge discharge residues)	Inorganic component – almost exclusively SEM Organic component – TLC, GC-MS/thermal energy analyser (TEA) also referred to as a chemiluminescent detector, GC, high performance liquid, chromatography with electrochemical detection (HPLC), capillary electrophoresis with and without MS and various combinations of MS including MS-MS with both positive and negative ion monitoring
Paint	Microspectrophotometry, FT-IR, SEM, pyrolysis GC, XRD, Raman spectroscopy
Surface distortions	Comparison microscopy, confocal microscopy, atomic force microscopy
Trace explosives	The same as for organic GSR analysis with the addition of XRD

Of the trace evidence types identified in Table I for all but one of the samples the amount of material that is recovered from a source is all that is available for analyses. This means that there is a severe restriction on the size of the sample that is likely to produce a positive identification. The same is not true for DNA analyses where the PCR technology enables multiple copies of the original DNA material present in a sample to be generated and a sample which, without replication could not perhaps have been successfully analysed, now becomes analysable. Scientifically, because we know the details of the processes involved, this is perfectly acceptable, but legally is there an argument that says we are not effectively analysing the sample that was recovered from the clothing of the suspect?

TECHNOLOGIES WHICH MAY HAVE AN IMPACT ON TRACE ANALYSES

Whilst already mentioned as a technique in Table I, Raman spectroscopy associated with Raman scattering, in its standard form has severe limitations in terms of its sensitivity especially where there is considerable interference from a fluorescent source. Recent advances have arisen with developments in laser technology and the possibilities of using resonance Raman scatter but also enhancement in the Raman signal arising from surface phenomenon referred to as surface enhanced Raman spectroscopy (SERS) so that strong signals can now be detected when the analyte is ad-

sorbed onto a rare metal colloid providing the analyte possesses a suitable chromophore. A combination of these two phenomenon of resonance and surface effects are referred to as surface enhanced Raman resonance scattering (SERRS) and will enable analytes to be detected at extremely low concentrations. Concentrations as low as 10^{-18} g have been detected for some analytes. Since many analytes of forensic interest can be linked to suitable chromophores e.g. DNA fragments, the SERRS technique has the potential for detecting many such trace materials. In respect of the latter such sensitivity would remove the requirement for the PCR reaction. Such sensitivity however, brings with it additional problems concerned with contamination.

Other major advances are likely to be in the association of electronic chip technology with biological systems. There are already models for re-useable microchips which are able to undertake the same type of analyses that are presently undertaken using PCR technology in the laboratory. Once these become perfected it will enable DNA profiles to be acquired at the scene and, with developments in telecommunications, distant databases will be interrogated and the suppliers of the biological fluid identified. Additionally, chromatographic and capillary systems have been etched onto microchips and this could also lead to crime scene analyses in the near future. This may mean that much of the present role of the forensic scientist will move from the laboratory to the crime scene and that the laboratory will be mainly concerned with the maintenance of databases.

However, the results of such analyses will still require interpretation and such interpretations will require to know the consequences of any possible contamination of the trace sample being analysed and precautions used to prevent contamination.

CONTAMINATION AVOIDANCE

Contamination is probably the most challenging problem that affects those involved in trace analysis work. The reasons for this are obvious since, if a trace sample which has been identified and characterised and is used to support a prosecution, is from a contamination source and not a true sample associated with the crime, then a miscarriage of justice will ensue. It is therefore vital that the forensic practitioner takes all precautions to avoid the possibilities for contamination and additionally is in a position to identify contamination when it has occurred. Moreover, unless steps are in place to eliminate contamination as a source of the evidence type then it becomes impossible to correctly interpret any analytical findings. To illustrate the problems of contamination consideration needs to be given to the following questions:

- Are those entering the crime scene and the trace laboratory properly attired and knowledgeable about the problems of contamination?
- Are the correct controls taken as a measure of possible sources of contamination?
- Does the packaging meet the appropriate contamination avoidance criteria?
- Is any transportation used for items or personnel contamination free?
- Are places of storage of recovered items free from contamination?
- Is the appropriate area of the laboratory to be used for analysis fit for purpose and especially is the entrance contamination free?
- Are all items and equipment taken into the laboratory contamination free?
- Does the laboratory maintain a programme of auditing for contamination by that trace evidence type?
- Is there full and up to date documentation that supports all these contamination avoidance measures?

INTERPRETATION OF THE ANALYTICAL RESULTS OBTAINED FROM TRACE EVIDENCE

Assuming that the analytical values obtained from the trace evidence type fall within the allowable range of values, that is, they fulfil the appropriate criteria under precision and accuracy, the values obtained must be interpreted for the courts against a series of criteria. These are:

- The situation surrounding the case – all case details as far as is possible must be available to the forensic scientist if he/she is to interpret their findings correctly.
- The use of data bases.
- Reports available in the scientific literature.
- The Scientists experience of similar cases.
- Scientific surveys of the occurrence of the trace evidence type in society.

In respect of assessing the evidence in the context of the case much will depend on the information provided to the scientist. If the scientist has attended the scene then he/she will be in the best position to formulate a hypothesis on the relationship between the trace evidence type and the series of events which are likely to have taken place at that scene. Where the scientist is dependent upon others in respect of the scene, then it is vital to for the scientist to obtain as much information as possible from the investigating officer. In this latter position, the scientist will formulate a hypothesis based upon the information supplied but if at a later stage in the proceedings fur-

ther information is brought to the attention of the scientist then this may call for a modification in the hypothesis he/she has been asked to formulate or it may be that an alternative hypothesis requires to be considered. It may also be at this stage that the scientist does not have sufficient experimental material or knowledge to test any alternative hypotheses. Depending on the stage through which the case is progressing in the legal processes such changes in interpretation may require modifications to any submitted report(s).

Databases are of vital importance if the forensic scientist is ever going to be able to correctly interpret his/her findings. However, it is important that databases meet correct criteria if they are to be of value. That is to say for databases to be of value they must be accurate in their information content, relevant to the trace evidence type and up to date. The first of these criteria must mean the implementation of a rigid quality assurance programme before any data is admitted to a data base. An important example are the DNA data bases that are being established at the moment. In respect of the second criterium, decisions must first be made as to the purpose of the database so that only data relating to that purpose is entered. For example, is the database to be used solely for casework, or is it to be used for intelligence purposes? Finally the database must be up to date if maximum use is to be made of its content. For example, with changes in fashion and developments in fibre technology would the database for fibre type and colour be different from what it was say two years ago? It should be recognised that the maintenance of any database can be expensive in terms of man-hours involved but this must be balanced against the benefits in evidence interpretation and the value they give to justice systems.

Reports given in the scientific literature mean that the forensic scientist must be up to date in his/her knowledge of developments in his/her particular forensic discipline but also those areas of the forensic sciences that are of general concern especially those dealing with evidence interpretation. No reputable forensic practitioner over recent years could have failed to have noticed the raft of papers dedicated to the application of Bayesian Statistics to the interpretation of scientific evidence especially those on glass and DNA evidence by Dr Ian Evett and his many associates. Traditional statistics may still have an important part to play as seen in the attempts to compare the pattern of results taken from the analyses of some swabs thought to contain explosive traces with those produced by simulation experiments using logistic regression analysis [9] but more recently Curran and co-workers [4] suggested the use of Hotelling's T^2 statistic, a multivariate equivalent of Student's t -statistic, for determining the match between glass fragments recovered from a suspect's clothing, and appropriate controls but a further paper by these same workers took these ideas and applied the Bayesian ap-

proach [5]. Another application of traditional statistics is an attempt to identify the source of glass through measurements of the trace elements found in such samples. A predictive model was developed using Fisher's linear discriminant analysis which was able, in most cases, to distinguish headlamp, container window, and vehicle float glasses [1]. However, it must be accepted that the Bayesian approach to the interpretation of trace evidence is perhaps the preferred one since it is the one which is most acceptable to the whole legal process and the ideas of the likelihood ratio (LR) remain dominant where:

$$LR = \frac{\text{Probability of the evidence if } C \text{ is true}}{\text{Probability of the evidence if } \bar{C} \text{ is true}} = \frac{P\langle F | C_i \rangle}{P\langle F | \bar{C}_i \rangle}$$

Those workers involved in these developments have placed the interpretation of evidence onto a sound scientific basis for the very first time. We cannot all hope to become statistically literate to the same level as such experts so we must rely on this new breed of forensic scientist to guide us.

Using the results of previously investigated cases can be of value in the sense they highlight the possible relevance of trace evidence types and where they may be found and the possible significance of any pattern associated with such evidence. However, care should be taken of using such knowledge because every case is unique and the absence of some feature in one case does not mean it is irrelevant when found in another.

Finally, surveys of the occurrence of trace materials in our society are important in assessing the coincidence probabilities. One type of such surveys relates to the occurrence of the trace target material upon say the clothing of those not involved in criminal activity. For this area there are reports for both glass fragments (Refs) and fibres [2]. The other relates to the findings of target materials at random in public places such as on public seats, on public transport, at airports and in police stations etc. There have already been at least two surveys of the incidence of fibres in society [6, 8] and one report of the finding of trace explosives [3]. This latter serves as a useful example.

For this survey samples were taken from taxis, buses, underground trains and stations, passenger aircraft and airports using either a swabbing procedure or vacuum recovery. A range of explosives were looked for including ethylene dinitrate (EGDN), the nitrotoluenes, nitoglycerine (NG), pentaerythritol tetranitrate (PETN) and RDX (C4). The findings are shown in Table II.

TABLE II. EXPLOSIVE TRACES FOUND IN SOCIETY

Site	Number of positives	Explosive found	Level range
25 taxis	3	RDX	5–18 ng
10 buses	0	–	–
2 airports	1	RDX	19 ng
2 aircraft	0	–	–
9 police custody	7	RDX	4 ng
Suits		NG	2–11 ng
19 police vehicles	9	RDX	12–111 ng
		NG	2–90 ng
		PETN	109 ng
Underground stations	33 (samples)	–	–

It is clear that traces of explosive can be present in society and the question must be posed as to their possible source. Certainly the taxis examined were closely involved with airport work and this was an airport from which army personnel were likely to embark. As a consequence army personnel are likely sources. This probably accounts for the single positive from the one airport. Traces of explosive were neither found on the two aircraft examined nor on the buses. The major source of explosive traces was the police custody suits and police vehicles. In most cases the explosive was nitroglycerine which suggests firearms as the most likely source of contamination. Just one police car was contaminated with RDX and PETN which suggest some specific source for this. Because the efficiency of transfer for explosives is low then the finding of very low levels from some examined sources should not give rise to concern although larger and more detailed surveys are required. It should be remembered that if contamination of suspects from police custody suits had occurred this should have been picked up through the controls taken at the time of sampling.

The finding of trace evidence materials especially on clothing must have arisen by some mechanism whether it be contact between two surfaces as would be normal for fibre traces or the projection from a point source such as might be found for glass fragments or by simple Brownian movement through the air as can occur with some fibres or through the shear volatility of the trace material as can be found with nitroglycerin explosive. Whichever mechanism is in place it is important to recognise that such exchanges do take place and that these may be exchanges in two directions but that they may also occur through secondary and sometimes tertiary transfers. In respect of these latter two, if such transfers are to occur they will often be dependent upon the amounts of trace material available in the first transfer and the efficiency with which the transfers occur. Many transfers are less than 20% efficient. No information appears to be available on the secondary

transfer of firearms discharge residues. It is important to develop knowledge of the persistence of trace materials on surfaces since if such items are lost very quickly from say clothing then there may be little point in looking for them. For example, the volatility of the explosive nitroglycerine means that it may be lost from a surface fairly quickly and if attempts are not made to quickly recover such material then inevitably no traces will be found. However, the explosive RDX can persist upon surfaces almost indefinitely unless something disturbs it so that its time of deposition may be impossible to identify. Another example would be firearms residues most of which disappear from the hands after 4 h [7]. This means that the time factor in the interpretation of the evidence is very important.

CONCLUSIONS

It is clear that we have gone a long way to discovering means of recovering trace materials from various matrices. Methods for their analysis have been and continue to be developed to detect smaller amounts with a high degree of certainty. We are now beginning to develop a rational framework for the interpretation of what these analytical findings may mean in the context of a criminal case. We still need to accumulate data to assist in interpretation research programmes. Financial support is required to enable these developments to continue and this may be the rate determining step in the development of our discipline.

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