

National Body Fluid Guidance Notes

1) Blood

1a. Examination for Blood

Searching for blood typically involves the combination of careful visual inspection aided by oblique lighting, low power microscopy, and a chemical screening test (presumptive test).

Bloodstains are usually red-brown in colour, however this can depend on the colour of the surface on which they are deposited and the conditions to which they have been subjected.

Likely bloodstains are tested using a chemical presumptive test for blood. If the stain contains blood then a colour change of the chemical reagent is observed. Therefore, if a visible red-brown stain that has the appearance of blood gives a positive reaction to a presumptive test for blood, the stain is identified as blood.

1b. Interpretation of Blood Pattern/Distribution (General)

When a person is bleeding, their blood may be transferred onto nearby surfaces. This can occur by a number of different methods, involving either direct contact or airborne transmission. The nature and distribution of the resultant bloodstain(s) on an item can help in evaluating how the blood staining arose.

Direct contact with a source of wet blood will result in a contact bloodstain or smear, whereas, spots of blood are from airborne transfer. Small spots of blood may be produced in a number of ways including from an impact into wet blood. Small spots of blood can also be seen when blood is expired from the airways such as a mouth or nose.

The amount and distribution of blood transferred will depend upon factors such as the nature and duration of the contact, the proximity of the people/objects involved, the amount of blood that was shed and whether a force was applied.

1c. Interpretation of Blood Pattern/Distribution (Assault)

During the course of a physical assault, if a person sustains an injury that bleeds, blood may be transferred onto the clothing of those involved, onto surrounding furniture and furnishings or onto any weapon(s) used. The appearance of a bloodstain and the distribution of groups of bloodstains may be used to ascertain how an item became blood stained.

When items stained with wet blood come into contact with other items a contact bloodstain results. If there is movement at the time of contact or whilst the blood is still wet then the resultant stain will be present in the form of a smear.

Large blood spots/ drips of blood are formed due to blood falling freely under their own weight whereas smaller spots are formed where energy has broken up the blood and dispersed it as small airborne blood drops of varying size, for example, when an object strikes a surface wet with blood, when blood is expired from a bloody nose or mouth, or when blood drips onto a surface already wet with blood. So when force is applied to wet blood, for example, if an injured area is repeatedly struck by a weapon or by punching or kicking, it is separated into small airborne drops which are dispersed away from their source and land on items that lie in their path. The blood spots formed by an object striking liquid blood are also known as impact spatter. The spots that result can have a characteristic shape and may be used to determine the approximate position from which they originated.

The amount and distribution of blood transferred will depend upon such factors as the nature and duration of the contact, the proximity of the people/objects involved, the number of parties involved in the incident, the availability of any blood to transfer and the degree of force applied, if any.

1d. Characteristic Blood Patterns

Kicking into an area wet with blood may produce a *characteristic* bloodstain pattern on the shoes and lower legs of the trousers of the kicker. This consists of contact bloodstains with associated airborne bloodstains radiating from the contact bloodstain(s). If a kick occurs into areas that are not blood stained, blood may still be transferred but not in a characteristic pattern or it may be that no blood is transferred.

Punching into an area wet with blood may produce a characteristic bloodstain pattern on the sleeves of upper garments. This consists of contact bloodstains around the cuff area with associated airborne bloodstains radiating from the contact bloodstain(s). If a punch occurs into areas that are not blood stained, blood may also be transferred but not in a characteristic pattern or it may be that no blood is transferred.

1e. Blood Patterns on Weapons

If a weapon, such as a baseball bat, had been used to strike a surface heavily stained with wet blood, contact bloodstaining and possibly blood spots radiating out from the contact bloodstain on the surfaces of the weapon may result. However, if the weapon struck a surface which is not bloodstained at the time then it is possible that no blood would transfer onto its surfaces. If the weapon is repeatedly used this can give rise to further characteristic bloodstains which are caused by the weapon being swung and striking a surface (cast off staining and percussive staining).

When a weapon such as a knife is used then blood is sometimes seen on the blade in a smeared pattern which, depending on certain factors such as the area injured on the body, may fatty deposits, skin and/or hair, although this is not always the case and depending on the circumstances of its use and what happens to the knife afterwards, there could be little or no visible blood on the blade.

1f. Absence of Blood

It is possible for someone to be the perpetrator of a physical assault and for no blood to transfer to them or their clothing. This could be because the attack took place at a time when there was no blood available for transfer (for example the injuries started to bleed after the assailant left, or the blood was absorbed by clothing preventing a transfer) or the attack took place to areas that were not blood stained (for example the victim was kicked about the body when it was a head injury that was bleeding).

1g. Altered blood Stains

Blood may undergo a change prior to forming a pattern which can affect its appearance, for example it may clot or become mixed with other body fluids such as saliva or urine. It may also become mixed with other non body fluids or attempts may be made to clean the blood by washing or wiping.

1h. Cast Off

Small airborne drops of blood can be produced if a blood stained object is moved rapidly, for example swinging a weapon. The blood is flung off the end of the object and this can produce a linear series of blood spots on the surface on which they land. Blood staining of this type is referred to as 'cast off' blood staining.

1i. Expired Blood

Blood can be propelled from the airway, for example, by coughing, sneezing or wheezing. The resulting pattern generally consists of a number of spots of blood of varying size some of which may be directional and some may have air bubbles present. The blood may also become mixed with other body fluids such as saliva or lung surfactant and can have a dilute or 'stringy' appearance. However, the absence of air bubbles, dilution or any stringy appearance does not necessarily indicate that a blood pattern is not expired blood and the pattern produced is dependent on the amount of blood present and the degree of force.

1j. Projected Blood

Blood can be projected from severed arteries and veins, often with some force, and can be deposited on nearby surfaces such as walls and clothing. This can result in the formation of a projected pattern of blood.

If an individual receives an injury that breaches an arterial or venous blood vessel, patterns of bloodstains can arise which are indicative of that type of injury. If the injured person subsequently moves projected patterns can arise which appear as circular bloodstains in line or in a zigzag or wave pattern. Projected patterns occur as a result of the blood being forced out of the breached vessel under intermittent pressure reflecting the injured person's heartbeat.

When a major blood vessel, such as the jugular vein, is severed, large volumes of blood can flow or pour out from the wound resulting in large areas of blood staining on any surface onto which the blood is deposited.

1k. Blood Trails

When blood falls onto a surface due to gravity alone, a large spot of blood (a drip stain) is formed. If the source of blood is moving, a drip trail is formed. If the source of blood is stationary, then several drops of blood will fall in one place. This can result in a pooling of blood and secondary spatter.

2) Semen

2a. Semen Persistence

Following a sexual act in which semen is ejaculated, the individual components of seminal fluid will remain in detectable amounts for different lengths of time. Either of two components are of significance in determining the presence of semen: one is a chemical called Acid Phosphatase (AP), and the other is the spermatozoa or sperm cells.

When semen is deposited in the vagina/anus/mouth during sexual intercourse, it can subsequently be recovered during a medical examination by sampling using swabs. The likelihood of detecting semen on vaginal/anal/mouth swabs and mouth washings is dependent on the delay between an act of sexual intercourse with ejaculation of semen and the taking of the samples at medical examination.

The current guidelines for sampling during the medical examination have been produced by the Faculty of Forensic and Legal Medicine and can be downloaded using the following link:

<http://fflm.ac.uk/publications/recommendations-for-the-collection-of-forensic-specimens-from-complainants-and-suspects-3/>

These guidelines are reviewed every 6 months to take into account new information and advances in forensic recovery and analysis. It should be noted that these guidelines are for the collection of samples only, taking into consideration the information available at the time of the medical examination. Any requirement for laboratory testing of the samples will be considered in light of the full circumstances of the case, using information provided on the medical examination forms and laboratory (MG21) submission form. It is not necessarily the case that all samples taken will need to be examined in order to progress the specific issues identified.

The detection of acid phosphatase on vaginal swabs is likely up to 24 hours after sexual intercourse.

Following ejaculation during oral/vaginal/anal intercourse, spermatozoa (sperm cells) are gradually lost from the mouth/vagina/anus. Spermatozoa can be detected:

- on mouth washings/mouth swabs taken within 12 hours, and more rarely up to 2 days after oral intercourse;
- on vaginal swabs taken within 3 days of vaginal intercourse, and more rarely up to 7 days and beyond;
- on anal swabs taken within 2 days of anal intercourse, and more rarely up to 3 days after intercourse.

In certain circumstances the spermatozoa may be absent or reduced in number and only the seminal fluid is detected. Vasectomy, medical conditions or natural causes may produce this stated and in the latter two it can be permanent or transitory. In the absence of spermatozoa, the presence of seminal fluid may be confirmed using the Florence Iodine test which detects the presence of the chemical choline which is present in seminal fluid.

In circumstances where semen could be mixed with other cells/body fluids, such as those that line the vagina or mouth or blood, attempts can be made to separate the spermatozoa (into a 'seminal' fraction) from the other cellular material (into a 'cellular' or 'epithelial' fraction) prior to DNA profiling. This is an attempt to avoid the production of mixed DNA results. However, this procedure does not always effect a complete separation, particularly if the proportion of semen in the sample is very low compared to the proportion of other cells. An incomplete separation of cells types can result in mixed DNA profiles being obtained. It should be noted that semen contains both spermatozoa and cells from the male donor.

2b. Examination for Semen on other items

Semen deposited into the vagina/anus during sexual intercourse will subsequently drain from these orifices onto items worn next to the genitals such as underwear. Semen can also be deposited on to an item of clothing in a number of other ways. These include direct ejaculation onto the item or by being transferred from a wet semen stained object or body part.

When semen is deposited on to an item it is usual for the acid phosphatase and the spermatozoa to remain in detectable amounts for an indefinite period of time. This is reliant on the clothing not being washed.

Semen stains on items such as fabric may sometimes appear as off-white, occasionally crusty staining, but are not always visible. The process of identifying semen on items of clothing or other such exhibits is a two staged process. In the absence of any visible staining, in order to locate them the item is initially screened for acid phosphatase, a component found in semen. If positive chemical reactions are obtained the area can be further extracted and the extract can be examined microscopically for the presence of sperm cells, which will confirm the presence of semen. However, it should be noted that positive reactions to this test may be obtained from other biological materials such as vaginal material, yeast and bacteria.

2c. Semen and Washing

If semen is deposited onto an item of clothing/bedding and the item is subsequently washed, obtaining a positive chemical reaction for Acid Phosphatase would be unexpected. However, it is known that sperm cells can be retained on an item after one or more wash cycles.

Furthermore, if a previously non semen stained item is washed with other items that are stained with semen, sperm cells may be transferred to the non semen stained item and may be detected. Therefore, the presence of trace levels of sperm cells on an item that has been washed may not be as a result of any sexual activity.

2d. Penile/Digital Penetration

If a penis/fingers penetrates the mouth/vagina/anus it is possible that saliva/vaginal material/faeces may be transferred to the surface of the penis/fingers. In addition, if any of these orifices are bleeding or have semen in them it is also possible that blood and/or semen from them may be transferred to the surface of the penis/fingers.

Cellular material/blood/semen/faecal material transferred to the penis can persist for up to 24 hours and more rarely up to 3 days. This transfer of material may also occur to fingers in digital penetration cases, although its persistence would likely be for a shorter period of time. Material transferred during such acts will be gradually lost for example via contact with items such as underwear, washing/wiping and further sexual or other activity.

2e. Condom Use

If a condom is worn correctly during vaginal/oral/anal intercourse with ejaculation then cellular material would be expected to transfer from the vagina/mouth/anus to the outside surface of the condom and for semen to be deposited on the inside. This semen/cellular material may persist on the surfaces of the condom for a considerable amount of time, depending on the conditions the condom is exposed to.

It is possible that whilst the condom is being removed and in any subsequent contact with it that semen and cellular material on the inside/outside surfaces may be re-distributed onto all surfaces of the condom.

3). Saliva

3a. Detecting saliva

The presence of saliva on an item or swab can be determined using a presumptive chemical test that detects an enzyme normally found in high levels in saliva called alpha (α) amylase, then extracting the stain to recover cellular material which could have originated from the mouth. There are no reliable tests to say specifically whether or not the cellular material has originated from the mouth, however, DNA profiling tests may help to determine from whom the cellular material could have originated.

Varying levels of alpha (α) amylase can also be present in other body fluids, but these are mostly at lower levels than in saliva with the exception of vaginal secretions and faeces. Therefore, the detection of alpha (α) amylase is not a conclusive test for saliva.

3b. Saliva transfer and persistence

Licking, sucking, kissing or biting may result in saliva being deposited onto the area which had been licked, sucked, kissed or bitten. The amount of saliva transferred will depend on such factors as the level of saliva production during this activity and the amount and duration of contact between the mouth and the skin surface. Saliva will persist on the skin for a short period of time as the staining will gradually be removed, e.g. by subsequent transfer onto the clothing being worn or by the action of washing or wiping. Saliva transferred onto clothing may persist until the item is thoroughly washed.

4). Wearer and Contact DNA

Cellular material may be deposited on the surface of an item when it is worn or handled. The amount of cellular material deposited will vary from person to person and also depends on other factors such as the cleanliness of the person's skin, the duration of contact and the nature of the item or surface. It is possible to sample this cellular material from areas that would be expected to have been in close contact with the wearer or handler, for example by recovering flakes of skin from the collar of a jacket, or by swabbing the handle of a screwdriver. The selected sample can then be submitted for DNA profiling analysis.

By comparing the DNA profiles obtained from the cellular material with those from reference samples, it may be possible to identify a potential wearer or handler of the item. In many cases this will be the regular wearer or handler of the item.

If an individual wears an item of clothing or handles an item for a short period of time they may leave little or no cellular material behind, and as a result an incomplete DNA profile or no DNA profile of that person may be obtained. However, if no DNA profile is obtained, it does not mean that a nominated individual did not, or could not have worn or handled an item. Furthermore, cellular material may also be removed by washing.

It is usually not possible to determine when DNA was deposited, or if a nominated individual was the last person to wear or handle an item. Therefore obtaining the DNA profile of an individual from an item cannot necessarily be related to a specific time or sequence of events.

5). DNA(STR) Profiling

5a. Introduction

A technique called STR ('Short Tandem Repeat') profiling has been used in this case. This is a form of DNA analysis. DNA is a complex chemical found in most cells of the human body. It carries genetic information that determines the physical characteristics of a person and controls the functioning of their body. The information is carried in coded form and half is inherited from each parent. Each person's total DNA complement is unique, although DNA profiling does not enable us to analyse an individual's total DNA. Instead, we look at certain areas of DNA which are known to have high levels of variability between people. In almost all instances, each person's DNA is the same in all of their cells so DNA recovered from blood cells will be the same as DNA from hair roots, saliva or semen.

5b. DNA analysis systems

DNA profiling uses an amplification technique to target and copy specific areas of DNA. In this case one or more of the following DNA analysis systems were used:

- DNA-17

Seventeen different areas of DNA are amplified. Sixteen of these areas contain STR regions. These are called D10S1248, VWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D22S1045, D19S433, THO1, FGA, D2S441, D3S1358, D1S1656, D12S391 and SE33. The

seventeenth area, known as amelogenin, indicates the sex of the donor of the DNA. Together the results from these regions are called the DNA-17 profile.

- SGM Plus

Eleven different areas of DNA are amplified. Ten of these areas contain STR regions. These are called D3S1358, VWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, THO1 and FGA; these areas are the same as the corresponding areas in a DNA-17 STR profile. The eleventh area, known as amelogenin, indicates the sex of the donor of the DNA. Together the results from these regions are called the DNA (SGM Plus) profile. All of the SGM Plus loci can be compared with their corresponding DNA-17 loci, but the additional information in the DNA-17 profile is not used.

5c. *Attributing a DNA profile to a particular body fluid*

When an identifiable body fluid is subjected to DNA profiling tests, it is *usually* the case that the DNA profile obtained, or certain DNA components within a mixed DNA profile, can be attributed to the identified body fluid. In some instances it is not possible to reliably attribute a DNA profile to a particular body fluid in this way, especially in weak samples.

5d. *DNA comparison*

A DNA profile obtained from a human body fluid such as blood or cellular material can be compared with the DNA profile of a given person. If the profiles are different then the DNA could not have originated from that individual. If they are the same, then that person, or any other person who has the same profile, can be considered as a possible source of the DNA. The significance of finding such a match may then be assessed.

5e. *DNA Mixtures*

A DNA profile which has originated from one individual usually consists of a maximum of two components for each region of DNA examined. The appearance of more than two components at any one of the DNA regions examined usually indicates the presence of DNA from more than one individual. DNA mixtures can comprise DNA from any number of contributors and may do so in any proportion. The more contributors there are to a mixed DNA profile the greater the number of components present. As the number of contributors increases the more likely it is that coincidental matches will occur. In order to statistically evaluate the significance of matching DNA components, the most likely number of contributors to the mixture must be taken into account.

If DNA from more than one person is present and there is more DNA from one person than the other(s) then the mixed DNA profile may present itself as a major contribution plus one or more minor contribution(s). A clear major contribution from one person may be compared to reference DNA profiles and, if a match is obtained, a statistical evaluation may be carried out. The remaining minor components may be collectively referred to as the 'minor' profile. Depending on the number of contributors to the minor profile, it may or may not be suitable for comparison to reference profiles.

When the proportion of DNA from two contributors to a mixture is more equal such that a major contribution is NOT discernible, it may not be possible to determine which components originated from which contributor, unless it can be assumed that the DNA of one contributor would be expected to be present due to known association with the item.

If there is a basis for assuming a clear contribution of DNA from two individuals then it may be possible to statistically evaluate the significance of matching results using standard methods. This is generally only feasible when there is a significant amount of DNA from at most two contributors and any other DNA contributions are at a far lesser level than the clear contribution of two people.

If there are more than two contributors to a mixed profile and/or when interpreting low level and/or low template DNA profiles, often referred to as complex DNA profiles, then the interpretation of matching results may not be so 'straightforward'. With some complex profiles it may only be possible to conclude that an individual can or cannot be excluded as a potential contributor.

Some complex DNA profiles, although unsuitable for standard statistical evaluation, may be suitable for advanced statistical evaluation using sophisticated software. Such software is not routinely employed in casework. In the absence of advanced statistical software, some profiles may be the basis of a subjective evaluation and an opinion offered in accordance with the Court Of Appeal Ruling R v Dlugosz, R v Pickering and R v MDS ([2013] EWCA Crim 2). Such an evaluation is based on the training and experience of the scientist and, unlike objective interpretations, has no statistical basis.

5f. Additional low-level DNA components

The sensitivity of current DNA profiling processes are such that it is not uncommon to detect additional traces of DNA in samples at a very low level. These traces can be considered to be commensurate with background levels of DNA and are not considered by the Reporting Scientist to have any bearing on the matter under investigation scientifically.

5g. Issues arising from the use of different STR kits in forensic investigations

The introduction of the DNA-17 short tandem repeat (STR) chemistries into forensic DNA testing in the UK has resulted in the use of several different STR kits. This, together with the continued use of legacy SGM and SGM Plus profiles, has raised some issues which should be considered in cases where profiles generated by different STR kits are being compared. The potential issues are listed here:

- **Incomplete comparison**

The DNA-17 systems test 16 STR loci (plus a sex marker), which include all ten of the loci tested in the previously used SGM Plus system. In cases where an SGM Plus profile needs to be compared to a DNA-17 profile, all of the SGM Plus loci can be compared with their corresponding DNA-17 loci, but the additional information in the DNA-17 profile is not used. Hence, the DNA comparison can be considered to be incomplete. If the source of the DNA is contested then it would be appropriate to attempt a more complete comparison, if possible.

- **Discordance**

All DNA-17 kits test the same set of STR positions (loci), ten of which are also shared with the SGM Plus system. However, there is a small risk that two different kits may give a different result for the same sample at one (or very rarely, two) of the loci tested. This risk is well understood and has been assessed in a large study carried out by the National DNA Database (NDNAD) using all test kits prior to their approval for NDNAD use.

1. *Apparent mismatch caused by discordance ('false negative')*: This form of discordance will most commonly be observed when a crime stain profile and a reference profile from the same individual have been tested either with two different DNA-17 kits or with a DNA-17 kit and the SGM Plus system. In these cases, discordance may be observed as a difference between the two profiles at one or (very rarely) two loci even though they originated from the same source. Such close matches between profiles will be identified, either by the NDNAD or by a scientist carrying out a direct comparison, and retesting of one of the two samples with the alternative kit will confirm whether this is a discordance event, or a genuine mismatch.
2. *Apparent match caused by discordance ('false positive')*: The potential for discordance between kits also raises the possibility, albeit very remote, that a subject could have a profile which appears to match a crime stain profile but does so only as a result of the different kits used. This form of discordance would require that all other loci in both the subject and crime stain profiles match by chance and so this possibility is, in almost every case, extremely remote. In fact, it is estimated that the overall risk of a chance match occurring, whether due to a random individual having the same profile as the subject, or due to a discordance event, is less than 1 in one billion (where full profiles are concerned).

6). Interpretation and evaluation of scientific evidence

6a. Forensic interpretation and evaluation

The forensic expert can have two different roles to assist in an inquiry; an investigator or an evaluator.

- *Investigative* opinion is usually given by the expert when at least one version of events is missing (e.g unconscious complainant, 'no comment' interviews etc). In these cases, the expert is unable to provide any weight or strength of support for any given proposition, normally due to the absence of sufficient information provided to them about the case circumstances and/ or the absence of sufficient information or detail about what each party states occurred. Occasionally, possible explanations may be generated by the expert, using their experience and expertise to attempt to account for observations/ test results, or to lead the investigation in a certain direction. The expert will not provide an exhaustive list of possible explanations, but, where appropriate, may describe what they believe to be a scenario which may be likely to generate the same or similar findings as seen in that particular case.
- *Evaluative* opinion is that based on case specific propositions, usually one of which is provided by the Prosecution and one provided by the Defence, and taking into account clear background information. With sufficient information, the expert may be able to provide a weight or strength of support that the findings provide for one version of events over another.

6b. Evaluation of the evidence

To help evaluate the weight of the scientific findings, the usual procedure is for the expert to consider a pair of relevant propositions based on the prosecution and defence positions and the issues in the case.

To evaluate the weight of any forensic result, the questions that the expert would address would be typically:

1. What is the probability/likelihood of obtaining this result (or finding) if the prosecution version of events were true?
2. What is the probability/likelihood of obtaining this result (or finding) if the defence version of events were true?

The *ratio* of these probabilities or likelihoods gives guidance on whether the scientific findings support one version of events over the other, and by how much.

It can be seen therefore, that, if either version is missing or is incomplete, the evaluation cannot be carried out, as only half the ratio would exist. Similarly if the prosecution or defence version of events changes or if details are added, the expert's expectation of obtaining the findings may change and hence the expert will need to re-evaluate the findings using the new versions of events.

6c. Scale of support

In expressing the evidential significance of the findings, the following scale has been used:

no, limited, moderate, moderately strong, strong, very strong, extremely strong support.