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Guidelines for Forensic Sampling of Scenes, Footwear and Vehicles for Soil and Vegetation

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1. Forensic Soil and Vegetation Sampling: general guidelines

1.1 Considerations

The primary principle is to obtain a true and representative sample for further investigation in a way that is transparent and fully recorded so it can be interpreted and repeated if possible. Soil samples must be handled carefully to avoid cross contamination or introducing any potential bias or artefact, e.g. contamination from plastics may affect organic marker analysis; cross contamination of samples; accidental loss of dried samples which may crack and fall from the source/questioned item; wet samples may damage paper bags; awareness of sample size; sample homogeneity; sample representation.

Depending upon the requirements of the police force carrying out the investigation, different people will be requested to take the samples.

Training in sampling with an experienced practitioner should be carried out prior to commencing work and a record of the training should be available.

The actual method used in each situation will depend on the specific case context. This protocol document is provided as general guidance.

If in doubt, please contact a forensic soil scientist to provide advice or to visit the site in person.

1.2 Health and Safety

Prior to carrying out the work guided by this document, please ensure that all persons have been properly trained.

Site, situation and sample risk-assessments should be conducted before collecting any samples. At the scene of crime, appropriate and approved personal protective equipment (PPE) is required and should always include safety spectacles, mask and nitrile gloves (double gloved). A Tyvek® coverall (or similar) with hood and shoe coverings should be worn. This is usually the responsibility of the Scene of Crime Officer, as well as any additional precautions they may ask for.

If any of the sources are suspected of bio-hazard or chemical (e.g. asbestos) or other substance known to be hazardous to health, an immediate risk assessment should be performed, the sources handled with mitigating measures in place, appropriate caution should be exercised, and appropriate labelling used.

Staff must have up-to-date immunisations against known soil-borne pathogenic organisms i.e. tetanus. Ensure that staff members are aware and trained in the use of the potential hazards of sharps (e.g. needle-stick injuries from dissection needles, forceps etc.).

Environmental conditions such as inclement weather or extremes of temperature or rainfall should be considered for possible influence on sampling and potential effects on samples as well as part of the general risk assessment.

1.3 Equipment

The minimum equipment required to carry out this protocol is as follows:

- Personal protective equipment (coveralls, disposable nitrile gloves etc.)
- High resolution camera and scale bar and identification labels for photographs
- Crime scene tape
- Location recording device
- Site recording form
- Unique sample identifiers
- Log book and pen
- Permanent marker pens
- Stainless steel spade and/or trowel
- Spatulas of varying sizes, or disposable plastic ones
- Disposable tooth brushes
- Measuring tape/yard stick
- Sample storage containers (sacks, vials etc.)
- Evidence bags
- Water (deionised)
- Virkon® (Antec International, Sudbury, Suffolk) or alternative sterilizing liquid (for cleaning equipment between samples)
- Paper towels
- Sample collection vials or pots with Teflon caps, or aluminium cap inserts.

Appropriate care must be taken to ensure that all equipment is thoroughly cleaned with known cleansing agents, that are fully recorded, before and after use to eliminate contamination and cross contamination, particularly when sampling more than one site.

1.4 Sampling Protocol

The following sections set out the description of sites, sample collection, and post-collection storage.

A check must always be made with the CSM and SOC officer/ scene examination team that any other sampling or collection has been completed for any agreed priority, alternate analysis e.g. footmark casting prior to securing the soil or plant material.

1.4.1 Site description

1.4.1.1 Photograph the site in question thoroughly, ensuring the characteristics of site vegetation (if any), aspect, and the environment are recorded. Take wide angled shots in four compass orientations.

1.4.1.2 Document any potential locations of interest such as blood staining or footwear marks and ensure appropriate recovery procedures for other forensic evidence have been carried out prior to soil collection.

1.4.1.3 Mark out the perimeter of the site with crime scene tape if this has not already been done (inner and outer cordon).

1.4.1.4 On the recording form, draw a map of the area including compass points and objects or features of interest; use a measuring tape to record distance or use other markers as reference points (e.g. edge of buildings) and record a Northing on images taken. Ensure highest resolution on the camera is used whenever possible.

1.4.1.5 Record GPS and map co-ordinates at the site if available.

1.4.1.6 If any sources (e.g. tools, footwear etc.) or items of interest have been discovered, mark these on the site map, or repeat steps 1.1 to 1.5 if these objects are in a different location.

1.4.1.7 Clearly assign unique identifiers using indelible marker to any samples to be collected.

1.4.1.8 Decide on the number of soil sampling points at the site to best represent the variation at that place, or other locations as indicated by prior investigation. These may include soil from disturbed ground; nearby sites for elimination from investigation; control or "background" samples; sites related to any stated alibi.

1.4.1.9 Reference samples should, as far as possible, be from the crime scene. For example, taken from under the same vegetation/slope/aspect as the area of interest. Similarities and dissimilarities should be recorded. Select a minimum of three points in each area and assign unique identifiers to these sampling points.

1.4.1.10 Once the site has been thoroughly recorded, and any other necessary scene of crime/police work has been completed, sample collection may begin.

1.4.2 Sample Collection

The following steps are required for the collection of samples at the scene. Please note that the details and staff involved in sampling will depend on particular overall procedures held within different police forces/agencies.

1.4.2.1 Each sample should be photographed in situ before recovery. The sample source may also be included in the recording of a wider scene investigation (see above).

1.4.2.2 A unique sample identifier must be assigned to each source using indelible marker.

1.4.2.3 Record descriptive and contextual details of the source in a log book or secure tablet.

1.4.2.4 Recover each source from the area under investigation. If a surface location, then take the topmost layer possible which may depend on the exact situation, vegetation and texture of soil. That is take the 0 to 0.5 cm or 0 to 1 cm depth of soil only, and over a surface area of about 10 cm by 10 cm as far as is possible to accumulate the equivalent of a large tablespoon of soil as a surface scraped sample and seal in a labelled sample pot, with the corresponding identifier, for transport. Staff must take care to avoid cross-contamination of sources, by using new gloves (remove outer pair each time a new sample is taken) and clean tools for each item (clean the tools with fresh deionised water, then in Virkon).

1.4.2.5 Soil samples selected at the scene under investigation should be collected using clean stainless steel tools (or strong plastic disposable ones if available, see 2.4, above) and stored in vials with Teflon® lids or another appropriate container. Certain containers are not suitable for storing samples as they may add contaminants which could affect laboratory analyses (e.g. plastic material in the lids of some containers may affect wax organic marker or VOC analysis). If plastic containers are used, a clean empty container should be provided to the analyst to use as an analysis blank.

1.4.2.6 Soil samples collected from the field (i.e. dug from the ground) should be recovered as appropriate to the investigation: e.g. if a pit has been dug to conceal an object, then it should be re-excavated and multiple samples should

be collected at regular intervals down the side wall of the pit (suggested a minimum of three replicates at each depth, but number will depend on site variability and other investigation clues and leads).

1.4.2.7 Collect the samples in order of increasing depth to avoid mixing soil layers: i.e. excavate the top soil first, and then take the sample before proceeding to the next soil horizon. The depths of these samplings should be recorded, and a photograph taken at each sampling point.

1.4.2.8 Aim to collect a minimum of about a tablespoon of soil at each sampling point to ensure scope for analysis. Enough soil must be collected to allow as many potential complementary laboratory analyses as possible – if in doubt, consult the James Hutton Institute forensic laboratory.

1.4.2.9 Vegetation samples should be collected in paper (or breathable) evidence bags, often collected in parallel, at the same location, as the surface soil sample. Take care if the samples are wet, that the integrity of the bag is not compromised.

1.4.3 Post-collection Storage

1.4.3.1 Return samples, in cooled containers if appropriate, to the forensic laboratory at the James Hutton Institute immediately, to reduce the possibility of sample degradation. If this is not possible, the samples should be stored in a dark cold place (e.g. a portable refrigerator or a cool box with ice-packs) until they can be transported to the laboratory.

1.4.3.2 Ideally, soils and vegetation should be transferred for examination and sub-sampled for analysis immediately after collection. If this is not possible, the samples should be stored in a secure, cool, dark and dry location such as a +4oC (fridge).

1.4.3.3 If soil samples are wet, they should ideally be dried at room temperature as soon as possible, in a secure environment, avoiding potential contamination. Deliver samples to the forensic laboratory at the James Hutton Institute as soon as possible and liaise with Prof Dawson.

2 Sampling Questioned Footwear, Tools and Other items of interest

2.1 Considerations

The primary principle is to obtain a sample for further investigation in a way that is transparent and fully recorded so it can be interpreted and repeated if possible. Soil samples must be handled carefully to avoid cross contamination or introducing any potential bias or artefact, e.g. contamination from plastics may affect organic marker analysis; cross contamination of samples; accidental loss of dried samples which may crack and fall from the source/questioned item; wet samples may damage paper bags; awareness of sample size; sample homogeneity (non-mixture and sampling of individual single source contact material); sample representation.

Training in sampling methods, and at least instruction in the sampling procedure, should be carried out prior to commencing work.

Depending upon the requirements of the police force carrying out the investigation, different people may be requested to recover the soil samples from any questioned items.

There may be differences between the heel and ball or toe or side of a shoe, depending on the weight and gait of the person who had worn the shoe and the environmental conditions (e.g. heavy rain, muddy ground).

Non-destructive visual observation of soil colour and composition may aid in deciding if the soil is from a homogenous source or there is variability that needs to be accounted for in sub-sampling.

A check must always be made with the CSM and SOC officer / scene examination team members that any questioned item has been sampled for any agreed priority alternate analysis e.g. human DNA or other biological analysis prior to securing the soil or plant material.

2.2 Health and Safety

Training and risk assessments should be carried out and personal protective equipment worn. See section 1.2.

2.3 Minimum Equipment

In addition to the equipment listed in 1.3 the following items are required for laboratory work:

- Glass petri dishes
- 1.5ml Eppendorf-style tubes
- Weighing boats and sterile wrap paper
- Scales, angled and straight
- Stainless Steel Dissection needle
- Small paintbrushes
- Stainless Steel forceps/tweezers
- Large metal or plastic tray
- Appropriate sampling sheet or benchkote® (GE Healthcare UK Ltd, Buckinghamshire)

2.4 Sampling Protocol

The following sections set out the description of sampling protocol, post-sampling storage and waste disposal. Note that the sampling protocol (Recovery of soil from exhibits) is usually carried out by a forensic soil scientist/ geoscientist.

2.4.1 Ideally the object should be sampled as soon as possible after collection. If this is not possible, it should be stored in a secure, cool, dark and dry location.

2.4.2 If the exhibit is not fully dry it should be allowed to dry slowly at room temperature (c.20°C).

2.4.3 Sample sources should be photographed in the laboratory, shown with a scale bar and sample identity label clearly visible. Record the photograph file names in the log book. The top, sole, sides and front and back of shoes should be photographed in sequence. The front and back of spades should be photographed and also the handle, if soil traces are seen. The inside of the shoe should be sampled after the exterior has been sampled.

2.4.4 Effective sampling from the sources on the item must account for sample heterogeneity. It may be necessary to harvest from several different points on the source (see Figures 1 and 2). In this case, treat each sampling point as a different sample. Drawings of the item should be carefully made, recording each sampling point spatially on the recording sheet (Appendix: Recording sheet).

Figure 1. Two sampling points on a shoe (red ellipses).

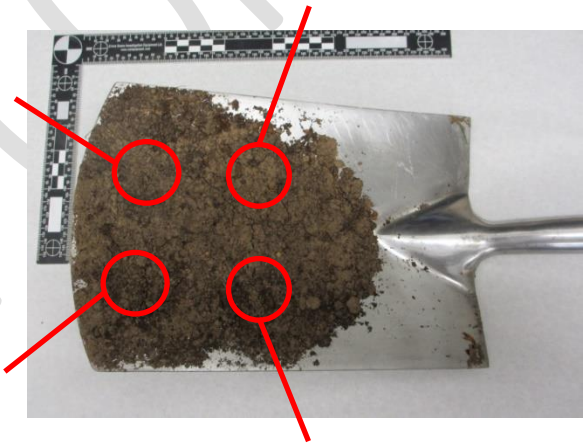

Figure 2. Four different sampling points on a spade (red ellipses).

2.4.5 Inspect each sample source and decide accordingly. Assign unique identifiers to each sampling point and associate them with the identifier of the source.

2.4.6 In some cases, there will be several layers at one position. In this case the layers should be carefully recovered separately and the position of the sample in the layering recorded.

2.4.7 In some cases, the source sample may be very small (see Figures 3 & 4). In such cases, recover as much soil as possible. It is helpful to place the source on a tray to collect crumbs of soil as they fall, and then recover these to add to the main sample if they appear to be similar in colour and consistency. Any soil of a different colour/texture is sampled separately. Ensure that the tray is clean and sterile before commencing sampling.

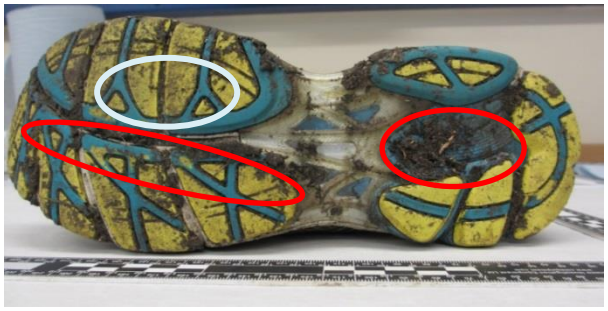

Figure 3. Red ellipses show sampling locations where substantial soil is present. Thinly deposited soil on a shoe is shown by the orange ellipse where a small amount is available).

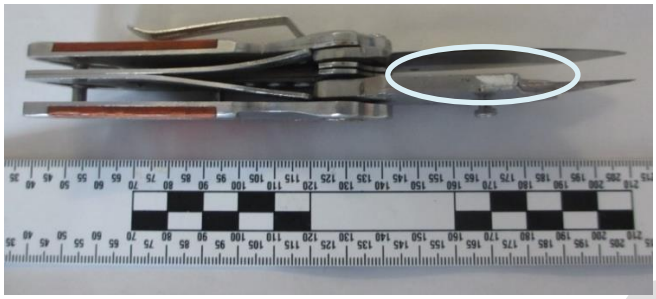

Figure 4. Blue ellipse shows small amount of material available for recovery from the knife.

2.4.8 Useful tools for recovering soil from exhibits such as tools and footwear include forceps, needles, spatulas and paint brushes. Ensure that all tools are cleaned and sterilised before each sampling. Carefully scrape soil from the source using whichever tool is most appropriate for the task.

2.4.9 Collect the soil particles in a glass petri dish or plastic pot. Individual aggregates should be collected separately as these may come from a source and aggregate structure can be informative. Soil scrapings should otherwise be mixed with a spatula to homogenise as thoroughly as possible. If a large amount of soil is available, sieve the sample through a sterilised 1mm sieve to aid with homogenisation.

2.4.10 Sample size required for various analyses is presented in Table 1.

Table 1. Guidance on sub-divisions of sample by type of analysis

Analysis	Minimum amount of soil required	Ideal Amount of Soil	Condition of Soil Required	Collection/ storage vessel
Soil Colour	0.1g	0.25g	Air dried	Plastic pot can be used (provided no inner sleeve in lid)
Wax Markers/ pollen	0.03g	0.25g	Air dried	Plastic pot can be used (provided no inner sleeve in lid)
Mineralogy/ elemental analysis	0.25g	0.5 to 3g	Air dried	Plastic pot can be used (provided no inner sleeve in lid)
Microbiology	0.1g	0.25g	Fresh, frozen	Eppendorf-style plastic microfuge tube

If the available soil is limited in quantity, consult with the forensic soil scientist to choose the most informative technique, based on expert knowledge of the soil type and general environment.

2.5 Post-sampling storage

The following steps are required for post-sampling storage.

2.5.1 Store soil samples for inorganic analyses, soil colour and organic wax markers at room temperature if dry. If wet, store in a 4°C fridge until they can be dried as soon as possible.

2.5.2 Samples of vegetation should be stored in the fridge if they are to be examined in the following two days. If longer than this time they should be dried at room temperature. On no occasion should wet vegetation samples be kept for several days as decay can occur and samples will not be able to be examined.

2.5.3 All samples should be labelled appropriately and reviewed regularly and as required. If in doubt about which method of storage to use, air dry the samples.

2.6 Post-sampling treatment

Please refer to the specific protocols for each analysis type as this information is not within the scope of this document.

2.7 Waste disposal

2.7.1 All samples are retained until the case is closed and for 30 years afterwards. Unless contaminated with biohazards, all non-chemical waste can be disposed of or recycled locally. Biohazard samples must be disposed via autoclaving (three times, with the temperature monitored and logged), and then can be sent to landfill. Chemicals and items contaminated with chemicals may be disposed of via specialist contractor, or as suggested by the CoSHH document.

2.7.2 Disposal of samples should be as instructed and agreed by the local police authority and noted in the archival records for auditing purposes.

3 Sampling Questioned Vehicles

3.1 Introduction

Depending upon the requirements of the police force carrying out the investigation, different people will be requested to recover the soil samples from questioned vehicles. This would usually be SOCOs carrying out any vehicle examination, but a forensic soil scientist can be requested to assist and/or provide advice.

The protocol in this section describes how to collect soil or vegetation found on or within a vehicle. Soil and associated trace evidence can be transferred to various locations both inside and outside of vehicles. The interior of the car, especially footwells and pedals, will often contain soil deposits.

The exterior of the car, especially wheel arches, tyres and the underside of the car should be closely inspected. The outside of the vehicle should be examined first and completed in a logical manner (i.e. Front Off side (FOS), Rear Off side (ROS), Front Near side (FNS), Rear Near side (RNS), Roof, Boot, etc etc) (Figure 4). Note if the car is right- or left-handed drive.

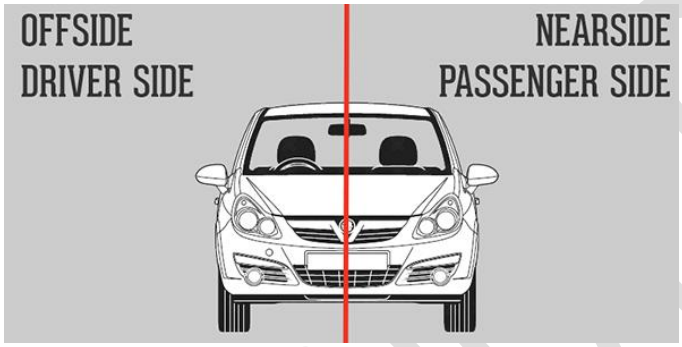

Figure 5. Offside and Nearside descriptions.

The examination and recovery from the inside should be carried out after the outside is completed.

Outside. Areas worth considering for taking samples are outside the boot area, wheel arches, tyres, under the vehicle (i.e. sump areas or areas near exhaust, where vehicles may have scraped along a track or exposed area in the centre of a country lane) (Figures 5, 6 and 7).

Outside soils can accumulate on wheel arches, usually in layers representing multiple depositions. These layers should, if possible, be sampled as separate samples as they likely represent different contact point locations. In addition, if the vehicle is a van, then consider runner boards as a contact source sampling location.

Figure 6. Splashed mud can be sampled to reflect contact with wet soil.

Figure 7. Mud flaps, exhausts and the underside of vehicles can yield soil and plant debris for subsequent analysis.

To examine underneath the car an inspection pit with good lighting is helpful. Vehicle air filters can sometimes retain material worth examining. The tyres themselves can sometimes yield soil and vegetation, although care must be taken in sampling these source materials, as there may be material embedded in the tyre from several locations.

Soils from separate sources often have a different colour or texture so can be visually distinguished when sampling; these samples should be taken carefully as separate samples. As well as recording which tyre the sample has come from, the position on the tyre should be recorded and mapped on a diagram, i.e. inside or outside, as inference may be made as to how the sample had become attached. Sometimes mud flaps and splashes can be useful sampling points as these can reflect travel through wet areas such as puddles along lanes etc.

Figure 8. Use of a sampling boat to collect discrete samples (in this case where a tyre had been sitting while the rest of the vehicle had been jet washed).

Interior. Soils can be found almost anywhere within the vehicle where someone has placed their feet, a tool or object and a secondary deposition of soil has resulted. Foot well samples are rarely good in terms of their evidential value as they are often composed of mixed sources. However, if discrete aggregates can be seen in the footwell, they should be sampled separately.

The inside of the boot of the car can sometimes have material that has fallen off a tool or spade used in digging.

Foot pedals are often useful sample locations as soil located there, likely reflects soil that has been transferred from a scene to the footwear of the driver. While valeting the remainder of the vehicle is often carried out after a crime has been committed, this area is often forgotten. If possible, pedal covers can be carefully removed and sampled from the vehicle intact and stored in a box for later examination in the lab.

Figure 9. vehicle foot pedals are possible contact points for soil transferred from footwear.

Consider soil smears on entry and exit points from the vehicle, and places where clothing, footwear or tools may have been stored, such as in the boot or passenger footwell etc.

3.2 Considerations

Soil (and the types of plants, spores and pollen that settle in the soil) can be characteristic of a particular area. When a person has driven to a scene or locus, they can take material both into the crime scene and out of the crime scene. Soil samples from car tyres or external regions of the car can be compared with soil taken from a crime scene. A 'match' or 'indistinguishable' profile may link (or exclude) a suspect with having been at the crime scene. It can also help direct police in search operations to areas that have the same or similar characteristics as the soil on the questioned item.

It is important to seize the suspect vehicle as quickly as possible after a crime has been committed, preferably prior to it having been used for subsequent travel.

If there are layers of soil, it is important to try to establish where the vehicle may have travelled before and after the suspected contact at the locus to ensure the layer most probably associated with the locus is identified and to have the most appropriate control samples collected.

If possible, samples (aggregates/peds) should be collected in separate single source sample layers. That is the case for both soil and vegetation.

3.3 Health and Safety

Ensure that all persons carrying out this protocol have been properly trained before commencing work and training is recorded. Perform a site risk assessment before collecting any samples. Consider staff immunisations against soil-borne pathogenic organisms.

Full Personal Protective Equipment (PPE) must be worn before approaching the seized vehicle. PPE consists ofsafety glasses or goggles, a face mask, gloves (doubled up), full crime suit, cuffs (or taped cuffs), hair net and foot covers. The top layer of gloves must be removed (and placed in a bag for

careful disposal) and replaced with a new pair of gloves before moving to another location on the vehicle, e.g. another wheel for sampling.

3.4 Equipment

The minimum equipment required to carry out this protocol is listed in 1:3 and 2:3. A large plastic sheet placed underneath the car to catch any falling debris would be useful. A container and bubble wrap would be ideal to transport the samples.

3.5 Sampling Protocol

The following sections set out the description of sampling protocol, post-sampling storage and waste disposal.

First, mark the position/location of examination (usually in police custody).

3.5.1 Vehicle description

3.5.1.1 Two people would generally operate together: one to photograph and to take notes, the other to describe and collect the sample.
3.5.1.2 Both people log the time and date and both sign the case book.
3.5.1.3 Record the name of the Crime Scene Manager and any other staff present, with time of entry and exit.
3.5.1.4 Take an image of the overall vehicle.
3.5.1.5 Take an image of the front, rear, offside (driver in UK) and nearside (passenger in UK). (FOS, FNS, ROS, RNS).
3.5.1.6 Take an image of the underneath of the vehicle using a secure ramp.
3.5.1.7 Decide on an appropriate sampling strategy based on where the soil and debris are present, working from outside of the vehicle inwards.
3.5.1.8 Only after the site details have been thoroughly recorded, and any other necessary scene of crime/police work, such as biological traces, has been completed, can sample collection begin.

3.5.2 Sample collection

The following steps are required.

3.5.2.1 **Each source should be photographed *in situ* before recovery.** The source may also be included in the recording of a wider scene investigation (see 3.5.1.6 above).
3.5.2.2 A clean plastic sheet or Benchkote should be used to place sampling kit on.
3.5.2.3 A clean plastic sheet should be placed under each area to be sampled to catch any soil or debris which might fall from that area.
3.5.2.4 **A unique sample identifier must be assigned to each source**, this to be recorded by the examiner and checked by the scientist recording in the log book.
3.5.2.5 Record descriptive and contextual details of the source in a log book, i.e., “offside, rear wheel, inner aspect, soil smear with fragments of vegetation”.
3.5.2.6 Recover each source from the area under investigation, and seal in a sample pot or a paper sack (depending on size and condition-see above for dry/wet samples, soil/vegetation), labelled with the corresponding identifier, for transport. Staff must take care to avoid cross-contamination of sources, by using new gloves and clean tools for each item (clean the tools with fresh deionised water, then Virkon or alternatively use strong disposable plastic tools).

3.5.2.7 Soil samples chosen at the scene under investigation should be collected using clean stainless-steel tools and stored in plastic/glass vials with Teflon lids or other appropriate container. Certain containers are not suitable for storing samples as they may add contaminants which could affect laboratory analyses (i.e. plastic inner lids on containers may affect organic marker analysis).

3.5.2.8 Try to collect single source samples as much as possible, i.e. soils of the same colour and texture. If a clump of soil with layers is removed, then try to keep the sample intact and pack carefully (e.g. in bubble wrap) for further sub sampling of the individual layers in the lab. Make a note of which is the outer (more recent deposit) and which is the inner layer (older deposit).

3.5.2.9 If plastic containers are used, a clean empty container may be given to the analyst to use as an analysis blank.

3.5.2.10 Remove samples to the laboratory immediately to reduce the possibility of sample degradation. If this is not possible, the samples should be stored in a dark cold place (e.g. a portable refrigerator or a cool box with ice-packs) until they can be transported to the laboratory.

3.5.2.11 If adhering vegetation is present and it appears wet, store in breathable evidence bags.

3.5.2.12 Samples for any biological analysis, i.e. DNA, should be frozen at -20^{o}C short term or -80^{o}C for long term.

3.5.2.13 All samples should be labelled appropriately and reviewed annually or as required. If in doubt about which method of storage to use, dry at room temperature.

3.5.3 Post-collection storage

3.2.3.1 Ideally, sources and soils should be sub-sampled for analysis immediately after collection. If this is not possible, the samples should be stored in a secure, cool, dark and dry location such as a +4^{o}C fridge, until appropriate sub-samples can be taken.

3.2.3.2 Soil samples should be dried at room temperature as soon as possible.

3.2.3.3 Plant samples should be examined fresh if possible, then air dried for subsequent storage.

Appendix 1: Forensic Science Examination Sheet

Laboratory Notes	Exhibit Number:	Case Number:

Examiner:	Date:	Time:	Room: