CHARCOAL

FORENSIC GUIDE
FOR SAMPLING,
EXAMINATION
& REPORTING

A guide for investigators and forensic experts seizing, sampling and/or reporting on charcoal shipments, aimed at those operating with limited equipment and/or proper equipment but with limited knowledge in terms of charcoal.

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Legal disclaimer
This booklet is intended to be a good practice guide for investigators seizing and/or reporting on illegal charcoal shipments, including those who may need to operate under suboptimal conditions.

This is not a training manual; it is a guide that assumes users have the relevant knowledge and training to seize, sample, analyze and report on charcoal shipments, and are aware of relevant national legislation concerning crime scene management, evidence collection and forensic reporting. In practicing the techniques described herein, it is the individual’s responsibility to ensure they have appropriate personal protective equipment, an understanding of the risks involved in attending a seizure of a charcoal shipment and prior knowledge of how to use the various equipment and materials suggested.

The authors and editors cannot accept any responsibility for any prosecutions or proceedings brought or instituted against any person or body as a result of the techniques described. All crime scene or evidence information represented, photographed or documented are fictitious.
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## CONTENTS

**ACKNOWLEDGEMENTS**

**1. INTRODUCTION**
1.1 HOW TO USE THIS GUIDE  
1.2 BACKGROUND AND AIM OF THIS GUIDE  
1.3 A SHORT INTRODUCTION INTO PLANT NAMES  
1.4 WHAT IS CHARCOAL?  
1.5 WHAT IS WOOD?  
1.6 WOOD ANATOMY  
1.7 CHARCOAL VS WOOD

**2. GATHERING EVIDENCE**
2.1 EVIDENCE COLLECTION  
2.2 EVIDENCE LABELLING AND STORAGE  
2.3 EVIDENCE DOCUMENTATION  
2.4 TRANSFER OF EVIDENCE/CHAIN OF CUSTODY

**3. CHARCOAL EXAMINATION USING CHARACTERISTICS OF SHAPES VISIBLE BY EYE OR LOW MAGNIFICATION**
3.1 SAMPLE PREPARATION  
3.2 METHODS OF VISUAL CHARCOAL EXAMINATION  
3.3 ANATOMICAL FEATURES OF INTEREST  
3.4 CLASSIFICATION

**4. FORENSIC REPORTING OF RESULTS**
4.1 NAME OR ID OF REPORTING INVESTIGATOR  
4.2 A UNIQUE REPORT NUMBER AND/OR TITLE  
4.3 THE QUESTION UNDER INVESTIGATION  
4.4 AN OVERVIEW OF THE SAMPLES THAT WERE INVESTIGATED  
4.5 INFORMATION ON WHERE AND HOW THE SAMPLES WERE TAKEN, AND ON THE CHAIN OF CUSTODY  
4.6 THE INFORMATION KNOWN TO THE REPORTING INVESTIGATOR OF THE SHIPMENT AND THE CIRCUMSTANCES  
4.7 THE METHOD USED FOR ANALYSIS AND THE RESULTS  
4.8 THE INTERPRETATION OF THE RESULTS AND THE CONCLUSION

**5. GUIDELINES FOR MORE ADVANCED ANALYSES**
5.1 WOOD/CHARCOAL IDENTIFICATION WITH HIGH MAGNIFICATION  
5.2 DNA ANALYSIS OF CHARCOAL

**ANNEX: CHAIN OF CUSTODY TEMPLATE**

**ANNEX: FORENSIC REPORT TEMPLATE**
INTRODUCTION
1.1 HOW TO USE THIS GUIDE

This guide is divided into five parts:

1. THIS INTRODUCTION
2. GATHERING EVIDENCE
3. CLASSIFICATION KEY FOR CHARCOAL
4. FORENSIC REPORTING OF RESULTS
5. ADVANCED FORENSIC EXAMINATION

They are presented in chronological order and should generally be performed in the order shown within this guide.

`At the beginning of each part a box outlines the rationale for undertaking the actions described. This rationale is intended to give you an understanding of why you are performing each action and thus help you use your initiative when alternative solutions are required.´

Permission to legally undertake some or all of these actions will depend on the applicable Standard Operating Procedures or protocols issued by your agency and the situation on the ground.

1.2 BACKGROUND AND AIM OF THIS GUIDE

Charcoal has been in demand in Somalia as a domestic cooking fuel for generations. Demand from cities and towns has grown, which has increased pressure on the bushland where charcoal is produced1. However, in the last decades the majority of the charcoal produced has been used as an export product2. In 2012 the UN Security Council adopted resolution 20363 that expressed "concern that the charcoal exports from Somalia are a significant revenue source for Al Shabaab and also exacerbate the humanitarian crises". Since then, export of charcoal from Somalia is under strict scrutiny and shipments of charcoal in this region are to be checked for their origin.

In their 1989 study, Bird and Shepherd state that the most active areas of Somalian charcoal production for Mogadishu are in the Bay region, where the bushland is characterized by scattered trees, mainly various Acacia species and other tree species such as Delonix sp., Dobera sp. and Terminalia sp., combined with a bush layer, woody vegetation and scattered grass cover. The wood of Acacia sp. and Terminalia sp. was reported to yield high quality charcoal and provided the main source for charcoal chosen for exportation.

The aim of this guide is to provide support to law officials who encounter possibly illegal shipments of charcoal and are required to write a report about their findings suitable for court. The chapter on the classification of charcoal is specifically aimed at charcoal from the countries in and around the Horn of Africa, so many plant species from other regions that can be used for charcoal are not included.

3UN resolution 2036 can be found at http://unscr.com/en/resolutions/2036
1.3 A SHORT INTRODUCTION INTO PLANT NAMES

Every language has its own set of names to distinguish and classify different plants. Given this variety, scientific names were developed to enable clear communication between people of different language backgrounds. Taxonomy is the branch of science that provides description, identification, nomenclature and classification of organisms, including plants such as trees. Trees can have many common names, but can have only one current scientific name. However, due to scientific progress in species classification there can be older historical scientific names, so called synonyms, for the same tree. Since 2005, most taxonomists agreed to split the large group of Acacia trees into an African group, now called Senegalia or Vachellia, and a much larger Australian group that retained the Acacia name. In this guide, the synonym Acacia is used as this name is more recognizable.

In general, scientific names for trees are used in this guide. The scientific naming of trees also represents the genetic closeness of common ancestors of the species. Different levels of ‘genetic closeness’ have different names. Plant taxonomists define the limits of each level (taxon), so that it is clear which plant species belongs to which levels. The relevant levels of this guide are, in order of increasing specificity: Clade, Order, Family, Genus and Species. For this guide, the key levels of analysis are: Genus and Species level, though sometimes Family level information is important. The levels higher than Clade are not used often and distinguish, for instance, between cone-bearing trees and flower-bearing trees or at higher levels between vascular plans, mosses and ferns. Note that Genus and Species names are traditionally printed in italics.

A translation of the most relevant plant names for charcoal, based (partly) on a study in 1990⁴, is given in Table 1 below. However, it should be noted that the same common names are often used for different scientific species, for example naming different species within the same Genus with one name. In addition, different regions can have different names or different spelling of names for the same tree species or different names for the wood and the tree it comes from. Therefore, the common names (English, Somali, Arabic) in Table 1 can only be used as an indication.

<table>
<thead>
<tr>
<th>FAMILY - SUB-FAMILY</th>
<th>GENUS (NEW)</th>
<th>SPECIES</th>
<th>ENGLISH</th>
<th>SOMALI</th>
<th>ARABIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabaceae - Caesalpinioideae</td>
<td>Acacia (Vachellia)</td>
<td>bussei</td>
<td>-</td>
<td>Galool</td>
<td>-</td>
</tr>
<tr>
<td>Fabaceae - Caesalpinioideae</td>
<td>Acacia (Senegalia)</td>
<td>mellifera</td>
<td>Blackthorn</td>
<td>Bilel</td>
<td>-</td>
</tr>
<tr>
<td>Fabaceae - Caesalpinioideae</td>
<td>Acacia (Vachellia)</td>
<td>reficiens</td>
<td>False umbrel-la thorn</td>
<td>Damal, Ghansa</td>
<td>-</td>
</tr>
<tr>
<td>Fabaceae - Caesalpinioideae</td>
<td>Acacia (Senegalia)</td>
<td>senegal</td>
<td>Cum acacia</td>
<td>Cadaad</td>
<td>Hashab, Alloba</td>
</tr>
<tr>
<td>Fabaceae - Caesalpinioideae</td>
<td>Acacia (Vachellia)</td>
<td>tortilis</td>
<td>Umbrella thorn</td>
<td>Qurac</td>
<td>Seyal</td>
</tr>
<tr>
<td>Fabaceae - Detarioideae</td>
<td>Brachystegia</td>
<td>sp.</td>
<td>Miombo</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Boraginaceae</td>
<td>Cordia</td>
<td>sinensis</td>
<td>Grey-leaved saucer berry</td>
<td>Mareer</td>
<td>-</td>
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<tr>
<td>Fabaceae - Papilionoideae</td>
<td>Dalbergia</td>
<td>microphylla</td>
<td>-</td>
<td>Dhuyac</td>
<td>-</td>
</tr>
<tr>
<td>Combretaceae</td>
<td>Terminalia</td>
<td>spinosa</td>
<td>Spiny desert tree</td>
<td>Xarar</td>
<td>-</td>
</tr>
<tr>
<td>Combretaceae</td>
<td>Terminalia</td>
<td>prunioides</td>
<td>-</td>
<td>Hareeri</td>
<td>-</td>
</tr>
<tr>
<td>Combretaceae</td>
<td>Terminalia</td>
<td>brownii</td>
<td>-</td>
<td>Hareri biins</td>
<td>-</td>
</tr>
<tr>
<td>Combretaceae</td>
<td>Terminalia</td>
<td>orbicularis</td>
<td>-</td>
<td>Bisaq</td>
<td>-</td>
</tr>
<tr>
<td>Combretaceae</td>
<td>Terminalia</td>
<td>polycarpa</td>
<td>-</td>
<td>Bisaq</td>
<td>-</td>
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<tr>
<td>Fagaceae</td>
<td>Quercus</td>
<td>sp.</td>
<td>Oak</td>
<td>-</td>
<td>Bilut</td>
</tr>
<tr>
<td>Pinaceae</td>
<td>Cedrus</td>
<td>sp.</td>
<td>(true) Cedar</td>
<td>Kedar</td>
<td>Saydar</td>
</tr>
<tr>
<td>Arecaeeae</td>
<td>Cocos</td>
<td>nucifera</td>
<td>Coconut (husks)</td>
<td>Qumbaha</td>
<td>-</td>
</tr>
<tr>
<td>Poaceae - Bambusoideae</td>
<td>various</td>
<td>various</td>
<td>Bamboo</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
1.4 WHAT IS CHARCOAL?

Charcoal is a lightweight black carbon residue produced by strongly heating wood (or other plant materials) in a low oxygen environment to remove all water and volatile constituents. Traditionally, in a process called charcoal burning, the heat is supplied by burning part of the starting material itself, with a limited supply of oxygen in a kiln. Charcoal burns with hardly a visible flame and without releasing much smoke. However, if the charcoal burning is less successful, only some of the wood is reduced to charcoal on the outside and the inside remains wood. The process of turning wood into charcoal destroys almost all other components of wood, but the morphological features remain intact.

1.5 WHAT IS WOOD?

Wood is a porous and fibrous structural tissue found in the stems and roots of trees and other woody plants. It is an organic material – a natural composite of cellulose fibers that are strong in tension and embedded in a matrix of lignin that resists compression. In a living tree, it performs a support function, enabling woody plants to grow large or to stand up themselves. It also conveys water and nutrients between the leaves, other growing tissues, and the roots. The term wood is also used to refer to other plant materials with comparable properties, such as coconut husks, and to material engineered from wood, or wood chips or fiber, such as plywood. In this guide, the term wood is used for plant material from stems or roots, and includes softwood, hardwood and bamboo, but not material from nuts or seeds, such as coconut husks.

Hardwoods are produced by trees that generally have broad leaves (Angiosperms). Hardwood from species that drop their leaves seasonally, such as oak, normally shows annual growth rings, but these may be absent in tropical hardwoods, such as umbrella thorn. Hardwoods have a more complex structure than softwoods and are often much slower growing as a result. Softwoods are produced by trees that generally have small leaves (Gymnosperms). Most species only drop leaves when there is insufficient water available and show growth rings related to environmental stresses, such as drought or rainy seasons. Hardwood trees generally produce harder wood than softwood trees, but there are significant exceptions. In both groups there is an enormous variation in actual wood hardness, with the range in density in hardwoods overlapping with those of softwoods; some hardwoods (e.g., balsa) are softer than most softwoods, while yew is an example of a hard softwood. Hardwoods are for example wood from *Acacia* sp., *Brachystegia* sp. and *Terminalia* sp. and softwoods is for example wood from *Cedrus* sp. or *Juniperus procera* (pencil cedar).
1.6 WOOD ANATOMY

Wood is composed mostly of hollow, elongated, spindle-shaped cells that are arranged parallel to each other along the trunk of a tree. The characteristics of these fibrous cells and their arrangement affect strength properties, appearance, resistance to penetration by water and chemicals, resistance to decay, and many other properties.

Just under the bark of a tree there is a layer of cells that grow and specialize to form bark tissue to the outside and wood to the inside. Newly formed and living bark transports the sugary sap down. Newly formed living wood (termed sapwood) conducts watery sap upward in the tree. Eventually, the inner sapwood cells become inactive and are transformed into heartwood.

In temperate climates, trees often produce distinct growth layers. These increments are called growth rings or annual rings when associated with seasonal growth patterns. Many tropical trees, however, lack growth rings, but some can have rings formed during droughts or other stressful times. The width of these rings varies according to environmental conditions and species.

When investigating hardwood, there are three morphological structures that can be used to identify at some taxonomic level the trees that formed it. Firstly, hardwood trees have specialized structures, called vessels, in the wood for conducting sap upward. Vessels are a series of relatively large cells with open ends, set one above the other and continuing as open passages along the tree trunk. In most hardwoods, the ends of the individual cells are entirely open; in others, they are separated by a grating. When looked at from above, vessels appear as holes and are termed pores. The size, shape, and arrangement of pores vary considerably between species, but are relatively constant within a species and so can be used to identify the tree that formed the wood. Along with the vessels most of the hardwood consists of smaller cells with wood fibers, which give strength to the wood. These smaller cells usually have small cavities and relatively thick walls. Thin places or pits in the walls of the small cells and vessels allow sap to pass from one cavity to another. The shape and size of these pits can also be used to identify the tree that formed the wood. Finally, there are strips of short horizontal cells that extend in a radial direction, perpendicular to the growth rings, called wood rays. These rays distribute sap horizontally through the tree trunk.

When investigating softwood, only pores and pits can be used for identification as vessels are absent. In contrast, bamboo is biologically not a tree, but a grass, and this is apparent in a different morphology. The stem consists of multiple vascular bundles within a spongy tissue with a hollow area inside. Each vascular bundle in bamboo contains two large openings for sap transport up, one smaller group of openings for transport down and solid material in and around the openings to give the vascular bundle and the whole bamboo stem strength.

1.7 CHARCOAL VS WOOD

Charcoal is commonly formed by burning wood in a restricted oxygen environment, such as a kiln. This incomplete combustion changes the chemical make-up of the components, but the anatomical structure remains largely unchanged. The classification of wood and charcoal can therefore be based on the same anatomical features, though some features may be distorted in size and shape.

Some features are easier to discern than others and some features are more informative than others. In this guide we will focus on features that are consistent and largely unaltered in charcoal formation, relatively easy to discern and are informative for distinguishing between relevant sources of charcoal. The morphological features mentioned here are based on the IAWA List of Microscopic Features for Hardwood Identification.
GATHERING EVIDENCE
GENERAL CONSIDERATIONS

Before any charcoal is sampled, you must be sure that all the documentation has been completed. In the eyes of the court, “if it isn’t documented, it didn’t happen”. For this reason, it is crucial that you take comprehensive notes before, during and after you are sampling. Describing what you observed is a very important form of documentary evidence and your notes will allow you to prepare accurate reports and statements at a later date. Such documentation may be crucial for presenting to a court an accurate recreation of the actions that occurred and led to the discovery and sampling of the charcoal. There are four steps to gathering evidence: collection, storage, documentation and transfer.

2.1 EVIDENCE COLLECTION

It is crucial that you know you have the authority to collect evidence before proceeding any further. It is advisable that if other types of forensic investigations are required, such as firearms investigations, in addition to charcoal investigation to discuss and optimize sampling strategies with the other forensic investigators beforehand.

2.1.1 AVOIDING CONTAMINATION

In general contamination is an important issue for forensic investigations. For morphological classification of charcoal contamination of the charcoal to be classified is less of an issue. However later analyses in the forensic laboratory might require the use of personal protection equipment during sampling. Note that charcoal should be handled with care to avoid further fragmentation.

2.1.2 HOW TO SAMPLE CHARCOAL

SAMPLING A LARGE SHIPMENT OF CHARCOAL

When sampling a large shipment of charcoal, several bags of charcoal have to be sampled randomly for later analyses. First, it must be established if the shipment consists of similar bags of charcoal based on visual characteristics and shipment paperwork. If the shipment consists of multiple distinct batches these have to be handled as separate groups (shipments) for sampling.

The necessary number of samples from the entire shipment, with similar bags, depends on the level of evidence required by the relevant law system. The sampling advice below is aimed at providing evidence that at least 50% of the bags in the shipment contain a certain type of charcoal. Following similar guidelines for drug shipments the following number of samples are advised to be able to report a conclusion with a 95% confidence level:

1. A minimum of five samples must be taken for investigation, if it is expected that all sampled units contain the same type of charcoal.
2. If re-sampling is not possible, eight samples are recommended. These eight samples are based on the possible finding that one of these samples cannot be classified or is classified differently than the other samples.
3. If the material gives rise to some doubt, at least eleven samples are recommended. This is based on the possible finding that two of these samples cannot be classified or are classified differently than the other samples.

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To randomly select the bags of charcoal for sampling, each bag can be assigned a number and a number can be randomly selected. Different methods are available to generate these random numbers, such as websites or functions in the Excel software. Aside from assigning each bag a number and randomly selecting a number, another option is to consider the three dimensions of the stack of charcoal bags and randomly select a bag within that stack. For instance, consider a stack of bags consisting of roughly ten by five bags stacked six bags high, with 300 bags in total. To select a random bag one random number can be generated for each dimension – length, width and height – so for this example stack a random number between 1 to 10, 1 to 5, and 1 to 6. A result of 7, 3 and 6 would indicate the 7th bag in the 3rd row on the top (6th) layer. In reality, bags are not stacked ideally on top of each other, but this method can be used as an approximation to select a random bag. For all methods, the chosen method to select random bags should be recorded in your notebook.

To sample an individual 25kg bag of charcoal, after selecting the bags from the shipment, one sample from each bag should be sampled and packaged separately. For optimal results, the bag should be opened, emptied on a sheet or tarp, and five to eleven of the largest chunks of charcoal collected. The remaining contents of the bag can then be returned to the bag.

In all cases, the charcoal sample should be placed in an appropriately sized, sealable evidence bag. The bag should be labelled and marked with a permanent marker pen as detailed in the next section.

### 2.2 Evidence Labelling and Storage

From the moment evidence items are collected it is important that they are stored in a way that minimizes any chances of contamination. Evidence can include physical items or digital items such as photographs. If evidence is not correctly stored the defense may try to introduce doubt and raise the possibility of contamination. This may result in the item of evidence becoming unusable for a prosecution. It is the responsibility of the person sampling to reduce the likelihood of such doubt.

You must always label and store individual evidence items in separate sealed containers or bags, even if they appear to have come from the same source.

#### 2.2.1 Labelling Evidence

Each item collected from a shipment of charcoal should be given an identifying number that is entirely unique. One tried and tested way of doing this is to combine the collector’s initials, the collection date and the number of items collected by that person on that day. For example, if it was the 16th piece of evidence collected on the 7 June 2019, by a person with initials ABC, the unique number may be 16ABC070619 (Figure 2.1). It is highly unlikely any other piece of evidence has this same code. When using this system, you must ensure that each evidence number is used only once by you each day, even if you attend multiple searches of shipments.

![Figure 2.1 Recommended method for generating a unique evidence identifying number](random.org, numbergenerator.org)
2.2.2 USE OF EVIDENCE BAGS

Evidence bags are printed with a unique evidence bag number that can be recorded in your notes and have space for information about the item and the chain of custody (Figure 2.3).

Details about the collection of the evidence item should be completed on the evidence bag. Not all fields need to be filled in if you do not have the appropriate information, but you should at least complete the following details:

- Collector’s name and signature
- Unique evidence item number
- Date and time of collection
- Location of collection
- Brief description of the evidence

While details about the collection of the evidence item should be recorded on the evidence bag, the “chain of custody” section – sometimes labelled “continuity” – must not be completed until the item is handed into someone else’s care (see 3.4 on chain of custody below).
If you make a mistake on an evidence bag, cross out your error and correct it. Then sign next to the correction to acknowledge the change. Record this change in your notebook (Figure 2.4).

Figure 2.4 Example of a correction to the date on an evidence bag, which has been signed to acknowledge its authenticity, and the accompanying notes regarding the change.

2.2.3 ALTERNATIVES TO EVIDENCE BAGS

While different types of evidence need to be collected in a variety of ways, all evidence collected should be placed into some form of packaging that can be "tamper-evident". Tamper-evident packaging is a way of storing something that cannot be accessed without damaging the package and thus making it obvious that it has been opened. Ideally such packaging would be a purpose-made evidence bag; however, if not available this can be substituted with an alternative. For most items of evidence, an envelope can be used for storage. The envelope can then be sealed using tape and the collector can sign their name across the openings so any attempt to open the envelope is obvious (Figure 2.5). Similarly, bin bags, shopping bags and paper can be used and sealed with tape.

If you are improvising with the evidence packaging, always ensure that the following details are recorded:

1. Collector’s name and signature
2. Name of the person who found the item on the scene
3. Unique evidence item number
4. Date and time of collection
5. Location of collection at the scene
6. Brief description of the evidence

Figure 2.5. Example of an envelope containing evidence sealed using tape with the collector’s name signed over the seal to make it evident if the envelope is opened.
Where packaging is not practical, for example very large items, the items must still be labelled (see Section 2.2.1).

Wet or damp charcoal should not be stored in plastic bags at room temperature for long periods of time as the charcoal may develop mold and hinder subsequent forensic investigation. As there is generally sufficient charcoal to select samples from, it is preferred to disregard the damp charcoal and select dry charcoal for sampling. Alternatively, package the wet or damp charcoal in a breathable material, such as a paper bag. Whatever method you have used to store the evidence, it is important to immediately document the details of the item and its collection in your notes.

2.2.3 ALTERNATIVES TO EVIDENCE BAGS

A written record of evidence collection and storage is essential. The documentation is likely to be used in court to explain what happened. Photographic documentation can be extremely useful to support your written record. The written record should be made as soon as possible after the evidence has been collected.

2.3.1 WRITTEN DOCUMENTATION

You should have already made detailed notes on each item of evidence that was sampled, as described in 2.1 and 2.2. During evidence collection you should ensure that you have also recorded the following information:

1. Which items of evidence have been collected
2. How they were handled
3. How they have been stored
4. All information written on the packaging/labelling
5. The unique evidence number for that item
6. The number of the evidence bag, if used

Once you have finished your work at a suspected charcoal smuggling location or shipment (crime scene), it is important that you prepare a written statement reflecting everything you have observed and done at the scene. The requirements for this written statement may differ depending on the judicial system of your country, however, in general, your written statement should contain at least the following information:

- Description and context of the crime scene
- Location
- Who was present and what their tasks were
- Your observations
- All actions taken
- Details regarding evidence and samples taken

You must sign and date your statement. All information in the report must correspond to your notes and any forms completed while processing the crime scene. All available information including notes, sketches, drawings or photographs can be requested for inclusion in the court files at a later date, so appropriate storage of these documents is essential.
2.3.2 PHOTOGRAPHIC DOCUMENTATION

All photographs taken as part of the investigation must be treated as crime scene records and stored appropriately. Even if photographs are not being included in your written statement, they must be stored in the event that they are requested for submission to court. Appropriate storage may be dictated by your agency, but the following are generally recommended for digital images:

- Transfer digital images to a dedicated data store, such as a hard drive, memory card or secure server.
- All photographs taken at the scene should be saved into a folder named with the case reference name and number, and labelled “originals”. This folder should not be opened by you.
- Make a copy of the “originals” folder and name this “working copy”, keeping the same case name and reference number.
- Store the “originals” folder safely on your computer or secure server or, preferably, transfer to a read-only disc.
- Once the “working copy” has been created, open it and confirm that all photographs have been properly saved.

Before using a personal device to take photographs, you should be aware that there may be a requirement to submit any device used to collect evidence to court, even if the data has subsequently been stored elsewhere.

2.4 TRANSFER OF EVIDENCE / CHAIN OF CUSTODY

The chain of custody is the unbroken series of records describing evidence storage, access and movement of the items from the time of evidence collection until presentation in court. The chain of custody is the chronological record of who had custody of the evidence at all times. It is of vital importance that each person who handles a specific piece of evidence records a statement of their actions.

Evidence is only admissible in court if it can be proven that it has not been tampered with between the crime scene and the courtroom. This is probably the most vulnerable part of the forensic process and is often scrutinized by legal representatives in court. Failure to prove that the chain of custody conforms to legal and professional requirements is likely to lead to rejection of the evidence.

The chain of custody must always be documented. Until you have correctly transferred a piece of evidence to another person, you remain responsible and accountable for that piece of evidence. There are two steps to the chain of custody:

1. Initial documentation
2. Transfer documentation

Both must be accurate and complete for the chain of custody to remain intact.
When a piece of evidence is first collected, the collector must have recorded how the evidence was collected and labelled. The collector is the custodian of that piece of evidence and is responsible for it until another person signs the documentation to confirm they are taking over custody. The transfer of evidence can be recorded on the evidence bag itself, which will have a pre-printed chain of custody section (Figure 2.6). In addition, separate chain of custody notes should be recorded and kept by those involved in evidence transfer, including the following information:

- Name of person releasing the evidence
- Name of the person taking custody of the evidence
- Signatures of both persons
- Date and time of transfer
- How it was received (in person/by post/taken from where?)
- Confirmation that the evidence packaging was intact

There is a chain of custody template at the end of this guide that can be used for this purpose. Alternatively, officers should record the transfer in their notebooks and both parties (those releasing and accepting the evidence) should sign and date each other’s notebooks.

When taking custody of a piece of evidence ensure that you understand and agree with all of the details documented concerning the evidence before signing, dating, and taking custody of the item (Figure 2.6). Check the last name on the chain of custody documentation is the same person you are receiving the evidence from, and that the evidence does not appear to have been removed from its packaging or tampered with in any way.

If a mistake is made when writing on an evidence bag or chain of custody form, cross out the error with a single line and correct it, but make sure to sign to acknowledge the correction. Record this change in your notebook.

Figure 2.6. Example of chain of custody documentation (“continuity”) on an evidence bag which has been completed by the recipient of the evidence to acknowledge transfer of custody.
CHARCOAL EXAMINATION USING CHARACTERISTICS OF SHAPES VISIBLE BY EYE OR LOW MAGNIFICATION
3.1 SAMPLE PREPARATION

Charcoal should ideally be examined in three sections: the transversal section (TS), the radial longitudinal section (RLS) and the tangential longitudinal section (TLS) (Figure 3.1). This is necessary because some features can only be seen in one or two sections and it also allows for the examination of the same anatomical features from different angles.

To examine the three sections the pieces of charcoal must be fractured. The fracturing can be done by hand using a razor blade or scalpel. When the charcoal has been fractured, the result should be a small cube of about 1 cm³ where all relevant sections are clearly visible.

To fracture charcoal, follow this step-by-step guide:

1. Select a piece of charcoal.
2. Saw off or break off a smaller piece that looks suitable for fracturing. Suitable pieces are those in which one plane, usually the transversal section, is clearly visible. This will make it easier to find the fracture lines for the other planes. Try to saw or break off the piece along the sections of interest, because this will make it easier to find the orientation for fractioning.
3. Align a razor blade or scalpel along the section of interest and apply pressure until the charcoal fractures. The goal is to achieve a clean break along the section of interest. Do not scrape the charcoal to create a flatter surface, because this will damage features of interest and create more charcoal dust which fills cellular features and obscures fine details. The correct orientation can sometimes be difficult to see, because charcoal is much darker than regular wood samples. Adequate lighting conditions as well as the use of a hand lens or magnifying glass can help in this regard.
4. Repeat step 3 for the other sections of interest.

Note: Fracturing charcoal can be challenging and requires practice. The extra effort put into creating a cleanly fractured section pays off in easier recognition of anatomical features of interest. Fracturing charcoal is ideally learned under the guidance of an experienced practitioner.
SELECT A PIECE OF CHARCOAL.

SAW OFF OR BREAK OFF A SMALLER PIECE THAT LOOKS SUITABLE FOR FRACTURING.

ALIGN A RAZORBLADE OR SCALPEL ALONG THE PLANE OF INTEREST AND APPLY PRESSURE UNTIL THE CHARCOAL FRACTURES.

3.2 METHODS OF VISUAL CHARCOAL EXAMINATION

Once you have a charcoal cube that has been fractured along all planes of interest it can be visually examined. In this guide we distinguish three levels visual examination:

1. Unaided visual examination
2. Low magnification
3. High magnification

Unaided visual examination is performed by examining charcoal with the naked eye, with the help of a small handheld magnifier or with the help of high-quality digital photographs. Examination with low magnification is performed with a reflected light microscope. Examination with high magnification is performed with a scanning electron microscope (SEM).

Examining charcoal at higher magnifications allows one to see more details of the anatomical features of interest. Some anatomical features can only be seen at higher magnifications. Increasing the level of magnification requires more expensive materials and deeper knowledge of the anatomical features on the part of the examiner. Examining more features allows for better classification of the charcoal and the exclusion of more alternative sources. In the next paragraph, the four most relevant anatomical features of interest that can be seen with unaided visual examination or low magnification will be discussed. The value in brackets is the number of that feature in the IAWA hardwood list (see 1.5)

Classification of charcoal based on anatomical features is best learned under the guidance of an experienced charcoal examiner as the identification of some features depends on the qualitative estimation of the observer. Whenever possible the classification of a piece of charcoal should be confirmed through comparisons with reference samples of the species and genera of interest, such as the species given in Table 1.
3.3 ANATOMICAL FEATURES OF INTEREST

1. PRESENCE/ABSENCE OF VESSELS

Hardwoods contain vessels for the transportation of water and nutrients along the length of the tree trunk and branches. In the transversal section the vessels appear as pores scattered across the surface. The presence or absence of vessels can be used to distinguish between hardwoods and softwoods. Acacia trees are hardwoods and therefore contain vessels. Some alternative sources of charcoal such as softwoods like cedar or the hard endocarp of the coconut do not contain vessels. Bamboo has visible vascular bundles that look similar to vessels but every bundle has two large pores.

This feature can be seen in the transversal plane at all levels of visual examination.

![Graphical representation of softwood, hardwood, bamboo and the woody part (endocarp) of the coconut. The bamboo section shows vascular bundles in the woody material and one vascular bundle in more detail. The coconut section shows the fibrous nature of the woody part of the coconut and the packed fibers in more detail. Note that the coconut has no longitudinal axis so only a cross-section is possible.](image)

2. WOOD DIFFUSE POROUS (IAWA 5. PRESENT)

In hardwoods, the size, distribution and changes in the number of vessels is also highly informative. In acacia species, the vessels have more or less the same diameter throughout the charcoal. Some alternative sources of charcoal, such as oak, ash and elm, are ring-porous, which means that vessels in early-wood are abruptly larger in early wood than in late-wood. In semi-ring-porous charcoal, such as walnut, pecan and hickory, the vessels are also larger in early wood, but the change is more gradual. Ring-porous charcoal is associated with temperate climates.

This feature can be seen in the transversal plane at all levels of visual examination (Figure 3.3).

![Graphical representation of diffuse porous wood and ring-porous wood.](image)
3. **PARATRACHEAL AXIAL PARENCHYMA ALIFORM TO CONFLUENT (IAWA 80-89)**

Paratracheal axial parenchyma is relatively thin walled tissue surrounding the vessels. In Fabaceae species the shape of the parenchyma, observed in the transverse plane, should have lateral (to the side) extensions (aliform) or form bands between vessels (confluent) (Figure 3.4). Other hardwood trees could have no axial parenchyma visible, or some tissue partly or wholly surrounding the vessels.

**Figure 3.4.** Transverse section of piece of Acacia sp. charcoal with paratracheal axial parenchyma indicated with the blue arrow. In this picture they are confluent and form wavy bands (white in the picture due to reflecting light). The grey lines in the background form a one centimeter grid.

**Figure 3.5.** Graphical representation of narrow same size rays, as found in Acacia sp., versus various options of different sized rays (these are not all present in one species).

4. **RAYS ARE NARROW (IAWA 97. WIDTH 1 TO 3)**

Rays are cells that radiate outward from the center and take care of the horizontal transport of water and nutrients. In acacia species all rays have approximately the same width of around three cells (Figure 3.5). Some alternative sources of charcoal, such as oak and beech, can have wider rays or rays of two distinct sizes.

This feature can be seen in the transversal plane at all levels of visual examination. In unaided visual examination rays appear as straight lines that radiate outward from the center of the wood (Figure 3.6).

**Figure 3.6.** Transverse section of piece of Acacia sp. charcoal with hardly visible rays of narrow width. The grey lines in the background form a one-centimeter grid. The lines reflecting the white light running from top to bottom are rays. These lines have a width of around three cells.

*Rays can also be used to determine the correct orientation for fracturing. Fracturing along the length of the rays will result in the radial plane. Fracturing perpendicular to the length of the rays will result in the tangential plane.*
The taxonomic level at which charcoal can be classified depends on the features that could be reliably identified and on the anatomical variation within and between the species and genera of interest. For instance, if only the presence of vessels can be determined then charcoal must have a hardwood source, which means that softwoods, bamboo and coconuts can be excluded. If the charcoal is also diffuse porous, then several temperate woods can also be excluded. Identifying additional features will allow for the exclusion of more and more alternative sources. Definitive species level classification of charcoal is usually not possible based on anatomical features, unless there is a clear either/or question between two sources of charcoal with known morphological variation. Distinguishing between common regional charcoal sources, such as *Acacia* sp., *Brachystegia* sp., *Terminalia* sp., etc., can also be challenging, because these genera share most anatomical features and high magnification is necessary (see 5.1).

For the anatomical features visible unaided or with low magnification the flowchart (Figure 3.7) can be used. The resulting classification is given in the green boxes.

**Figure 3.7.** Flowchart of classification process using the features from 3.3. The green boxes indicate the possible classification.
4

FORENSIC REPORTING OF RESULTS
After sampling the shipment suspected of containing illegal charcoal and analyzing the samples, the next step is reporting the results. The different tasks of collecting the samples, analyzing the samples and writing the forensic report can be performed by the same person, but these tasks can also be performed by different persons as long as the chain of custody is maintained.

A forensic report should contain at minimum:

1. The name or ID number of the reporting investigator
2. A unique report number and/or title
3. The question under investigation
4. An overview of the samples that were investigated
5. Information on where and how the samples were taken, and on the chain of custody
6. The information known to the reporting investigator of the shipment and the circumstances
7. The method used for analysis and the results
8. The interpretation of the results
9. A final conclusion that answers the question

### 4.1 Name or ID of Reporting Investigator

*In general, legal systems require that the person reporting the results of the investigation can be identified and is available to be questioned directly. In some legal systems it is required that the reporting person present their findings verbally in court. Check with your local legal system for the requirements.*

The name or ID number of the reporting investigator should be clearly present on the report. It should also be clearly stated which parts of the report the reporter investigator is responsible for, and preferably who or what agency is responsible for the other parts.

*To prevent an accidental mix-up of reports in the legal system it is advised to give the report a unique title and combine it with an unique number if possible. Especially if multiple reports by the same reporting investigator end up in the same case file.*

### 4.2 A Unique Report Number and/or Title

An unique report number can be generated in the same way as for pieces of evidence (see 2.2.1): the reporting investigator’s initials, the reporting date and the number of reports finished by that person on that day can be combined. For example, if it was the 2nd report finished by a person with initials ABC on the 20th of June 2021, the unique number would be 02ABC200621.

For a more specific title, the date, location and type of shipment can be included. For example, the title could be ‘Charcoal investigation of shipment seized on 4th of June 2020 from vessel A at harbor Y’.
4.3 THE QUESTION UNDER INVESTIGATION

Preferably the forensic question that is being investigated is known before analysis starts. This will ensure that potential bias is limited. By formulating the question together with the person legally in charge of the investigation, such as a prosecutor, the question will be better suited to the specific case in the court room. This in turn will provide better information to the court.

In general, questions about the legality of a shipment or legality of the actions of the suspect are decided by court, so should not be part of the question in the forensic report.

The question that is being investigated in the report should be written in the report. Questions can be about the classification of the material in the seized shipment, but can also be more complex and ask about the possible origin.

For forensic charcoal investigation these are some example questions linked to issues of classification:

- What kind of charcoal is in the seized shipment?
- Is the charcoal in the seized shipment from acacia trees?
- What material is in the seized shipment?

Forensic questions on possible origin in general deal with two different propositions: One from the prosecutor and one from the suspect. For forensic charcoal investigations, these are some examples of such propositions:

- The shipment contains some charcoal from the Federal Republic of Somalia vs The shipment contains only charcoal from the Republic of Kenya.
- The shipment contains some charcoal from acacia trees from the Federal Republic of Somalia vs The shipment only contains charcoal from cedar trees from the Lebanese Republic.

The exact wording of the propositions will impact the conclusion than can be drawn from the result. For instance, when considering the first pair of propositions, the result of classifying the seized charcoal as acacia charcoal will only be informative if Kenya does not export this type of charcoal. When considering the second pair of propositions, the same result (acacia) will exclude cedar trees and these results will strongly support the first proposition.

Note that two propositions can only be properly evaluated if they cannot both be true at the same time, which is why the terms “some” and “only” are included. In addition, some results exclude both propositions, which means these propositions cannot be evaluated and new propositions are necessary. For example, if the results indicate the shipment does not contain charcoal the example propositions cannot be evaluated.
4.4 AN OVERVIEW OF THE SAMPLES THAT WERE INVESTIGATED

For the reader to understand the conclusion of the report, she/he must also know which samples were investigated and which were not. Discrepancies between a sampling report and the classification report will also become clear to the reader and the responsible person can be asked for clarification. For instance, if 20 samples were taken, but only 10 were received for investigation, then questions regarding the selection of samples should be answered by the person sending the samples for investigation and not the (reporting) investigator. In contrast, if 20 samples were received but only 10 were investigated, the question on how they were chosen should be answered by the (reporting) investigator. Expected questions on these issues should, preferably, be answered in the report beforehand.

A list of all the samples that were received for investigation should be included in the report. All samples should have a unique identifier and, if not assigned one yet, one should be assigned before the investigation continues. If some samples were not investigated the reason for doing so should also be included at this point.

4.5 INFORMATION ON WHERE AND HOW THE SAMPLES WERE TAKEN, AND ON THE CHAIN OF CUSTODY

A secure chain of custody is needed to ensure that the samples under investigation are the same as the ones seized from the suspect. Doubts about the chain of custody can endanger the legal proceedings. If the chain of custody cannot be established at the start of the classification, it is advisable to check with the person in charge of the legal proceedings if they will be able to use the results in court or not. If results are not admissible in court, the classification will not be useful and limited investigation time can be spent elsewhere instead.

In addition to the overview of samples, information should be added on how and when the samples were received and who handed them to the laboratory or the person doing the investigation. This is part of the chain of custody necessary for forensic investigations.

4.6 THE INFORMATION KNOWN TO THE REPORTING INVESTIGATOR OF THE SHIPMENT AND THE CIRCUMSTANCES

Information on the background and the circumstances are sometimes useful for the interpretation of the results. Sometimes, if not mentioned that certain information is used, it can unknowingly be used multiple times as evidence in one case. By adding it to the report, the reader is aware of what the reporting officer has considered. Also, it is possible that afterwards the information is found to be inaccurate. By including the information and the source in the report, it is easier to detect which reports might need revision and which are unaffected.

In addition to the overview of samples, information should be added on how and when the samples were received and who handed them to the laboratory or the person doing the investigation. This is part of the chain of custody necessary for forensic investigations.
4.7 THE METHOD USED FOR ANALYSIS AND THE RESULTS

By providing the method and how it was applied to the sample to produce the reported result, it is both clear to the reader what the results are based on and also clear to other investigators if a valid method was used and applied correctly. Clear forensic reports help the court understand the value of the results.

A description should be added of the method used and the result produced for every sample that was investigated. If morphological keys, such as in this guide, are used for classification, both the key and the features used that lead to the result should be mentioned, as well as the result itself.

For example: The classification key in the guide [name of guide] was used to classify sample XYZ. Based on the features of [feature 1], [feature 2] and [feature 3] this sample was classified as [result of key].

4.8 THE INTERPRETATION OF THE RESULTS AND THE CONCLUSION

This part of the report should guide the reader to understand how the reporting officer used the findings to get to the conclusion. When done correctly, reading the conclusion should not come as a surprise to the reader.

In this part of the report, you explain to the reader what the results mean with regards to the investigative questions. This can consist of both a statistical interpretation and a criminalistic interpretation. The statistical interpretation is based on the chosen sampling procedure and the number of classified samples. An overview is given in Table 2.

**TABLE 2 OVERVIEW OF SAMPLING PROCEDURES, ANALYSIS RESULTS & STATISTICAL INTERPRETATION**

Number of samples refers to the chosen sampling strategy. The number of classified samples are the samples where the classification yielded the same results. If multiple samples yield different results, the interpretation can be used for each group of similar samples. If all the charcoal analyses yield no results, for instance due to bad quality of the material, the result is 0 classified samples. In the interpretation column, the [classification] should be substituted with the relevant classification result of the analysis.

<table>
<thead>
<tr>
<th>NO OF SAMPLES</th>
<th>NO OF CLASSIFIED SAMPLES</th>
<th>STATISTICAL INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>LESS THAN 5</td>
<td>0</td>
<td>EITHER THE CHARCOAL IN THIS SHIPMENT CANNOT BE CLASSIFIED. OR POSSIBLY THERE IS NO CHARCOAL IN THIS SHIPMENT.</td>
</tr>
<tr>
<td>LESS THAN 5</td>
<td>AT LEAST 1</td>
<td>PART OF THE CHARCOAL IN THE SHIPMENT CONSISTS OF [CLASSIFICATION]</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>EITHER THE CHARCOAL IN THIS SHIPMENT CANNOT BE CLASSIFIED. OR POSSIBLY THERE IS NO CHARCOAL IN THIS SHIPMENT.</td>
</tr>
<tr>
<td>5</td>
<td>LESS THAN 5</td>
<td>PART OF THE CHARCOAL IN THE SHIPMENT CONSISTS OF [CLASSIFICATION]</td>
</tr>
<tr>
<td>5</td>
<td>5 (ALL)</td>
<td>MORE THAN 50% OF THE CHARCOAL IN THE SHIPMENT CONSISTS OF [CLASSIFICATION] (95% CONFIDENCE LEVEL)</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>EITHER THE CHARCOAL IN THIS SHIPMENT CANNOT BE CLASSIFIED. OR POSSIBLY THERE IS NO CHARCOAL IN THIS SHIPMENT.</td>
</tr>
</tbody>
</table>
For forensic charcoal investigations that require classification the statistical interpretation of Table 2 is sufficient to proceed to the conclusion.

Some forensic charcoal investigations also consider propositions on origin. For these investigations other types of information that are available to the investigator and relevant for the forensic question should be considered and added to the interpretation part of the report. In particular, for each proposition under consideration, all the findings should be listed and for each finding noted if and how well it fits the proposition. Subsequently, these lists can be compared and evaluated to interpret the findings considering the pair of propositions. For an introduction on how to report these types of evaluation the ENFSI guideline\(^7\) is recommended.

For example, there are two propositions:

‘The shipment contains some charcoal from acacia trees from the Federal Republic of Somalia’ versus ‘The shipment only contains charcoal from cedar trees from the Lebanese Republic’.

The findings are more than 50% of the charcoal in the shipment consist of hardwood of Fabaceae trees.

Relevant information that can be gathered is 1) Almost no Fabaceae trees in the Lebanese Republic are used or exported for charcoal, 2) Acacia species are members of the Fabaceae family, 3) Acacia is often exported as charcoal from the Federal Republic of Somalia.

The findings fit the first proposition very well, considering information No. 2 and No. 3. For the second proposition and considering information No. 1, the findings would only fit this proposition if this shipment was a rare exception. To express the value of this evaluation, the evaluation of “fits very well” is compared to “rare exception”; we could therefore conclude that the forensic findings provide strong support for the first proposition rather than the second proposition.

5

GUIDELINES FOR MORE ADVANCED ANALYSES
Laboratories performing the analyses described in this chapter often have a quality assurance/quality control system in place. Adhere to these systems and employ relevant controls. Consider analyses of results by two individuals (the four-eyes principle) or the peer review of data and/or casefiles as standard procedures.

5.1 WOOD/CHARCOAL IDENTIFICATION WITH HIGH MAGNIFICATION

The principles for identifying charcoal at high magnification are the same as for lower magnifications—recognizing anatomical features of interest and comparing these to known sources of interest. Examining charcoal at higher magnification allows for easier recognition of more anatomical features for more definitive identifications at lower taxonomic levels. It should be noted that charcoal identification is generally limited to genus level, because there is little variation in anatomical features within genera. Identification at genus level also places additional requirements on the knowledge of anatomical features of interest on the part of the examiner and the availability of reference materials for comparison purposes.

5.1.1 HIGH MAGNIFICATION (USING A SEM)

Most features can be seen with both a reflected light microscope and a scanning electron microscope (SEM), but SEM has a number of additional benefits. Charcoal can be difficult to examine with reflected light microscopy, because it is dark and uneven material. The use of a SEM alleviates some of these issues. With SEM the sample is scanned with a focused beam of electrons that results in clear images with a high depth of field that is not hampered by the darkness of the material. The higher magnifications possible with a SEM also means that features of interest can be examined in greater detail to ease identification (see Figure 5.1). Drawbacks of SEMs include the high initial cost and the requirement of specialized knowledge to use and maintain the equipment, though the development of tabletop-sized SEMs has lowered costs and has increased ease-of-use considerably.

5.1.2 REFERENCE MATERIAL

Wood anatomical characters are described according to the standard terminology of the International Association of Wood Anatomists (IAWA). Identification to the appropriate taxonomic level can be aided by comparison of unknown samples to published descriptions in scientific papers, (tropical) timber atlases and computerized databases both online and offline.

These comparisons are greatly facilitated by access to a collection of reference material, preferably of charcoal samples. To determine which plant species should be added to a collection of reference material depends on the forensic questions, such as 'What alternative charcoal sources do we expect to encounter?'. Table 1 in Chapter 1 provides a short list of plants that could be used as a starting point. In addition, variation within a species should be considered by collecting multiple samples from different individual plants from the same species. Finally, variation between the same species growing in different localities or environments can also be of interest for the collection. Building a reference collection by collecting and describing samples is a good way of learning to classify charcoal and can be used as training to become proficient in this method.

Reference data, as opposed to reference material, refers to the results of previously analyzed reference material, such as macro- and microscopically photographed charcoal or wood (timber). When using reference data from external sources, it is important to carefully consider the source and reliability of the data. Questions that should be considered include ‘Can the results be traced back to taxonomically validated reference material?’; ‘Have the methods used been peer reviewed and published?’ and ‘Have the performed analyses been thoroughly explained and justified?’. Preferably, external reference data should only be used after an internal validation study of that data has been performed.
5.2 DNA ANALYSIS OF CHARCOAL

Vascular structures such as those found in wood only contain low quantities of DNA. When wood is charred, the little DNA that was present is badly degraded or even destroyed altogether. Additionally, charcoal is a brittle and porous material that may contain exogenous DNA. Such DNA may interfere with analyses, especially when this DNA originates from another botanical source such as pollen from flowering trees.

The analysis of charcoal DNA therefore requires specific facilities, procedures and personnel in order to:

- Select the most suitable charcoal samples for DNA analyses
- Remove potential sources of exogenous plant DNA prior to analyses
- Utilize techniques designed and validated for the analysis of trace amounts of degraded DNA
- Prevent contamination of samples, reagents, consumables, etc. with (plant) DNA
- Prevent contamination of samples, reagents, consumables, etc. with previously amplified DNA (strict separation of pre- and post-PCR processes, aliquoting of reagents, etc.)
- Have measures in place to monitor contamination

Failure to adhere to such procedures may result in analysis of contaminating or exogenous DNA, potentially resulting in incorrect and misleading conclusions.
Analysis of DNA from charred wood can be successful, especially when charring is incomplete. However, due to the harsh conditions created in the charring process, results are not always obtained. Failure to obtain sufficient DNA of sufficient quality does not necessarily indicate failure to perform the correct laboratory procedures.

To date, no studies have yet been published on the success rate of DNA-based identification of charred wood. It is expected to be lower than the success rate of DNA-based identification of processed traditional medicines (0-20%).

5.2.1. SAMPLE SELECTION

OBJECTIVE

Select those fragments of charcoal with the highest probability of containing sufficient original DNA for analysis and take (at least) two subsamples for DNA extraction.

RATIONALE

The surface/exterior of pieces of charcoal is more often contaminated with exogenous sources of DNA and is therefore generally less suited.

Partially charred pieces of charcoal (brownish) potentially contain less degraded DNA that fully charred pieces of charcoal (black)

PROCEDURE

- Inspect the pieces of charcoal that are available for analysis. (Figure 5.2.1)
- If present, select several pieces that are large enough to remove the outer layer. Splice the selected pieces, to assess the interior of the piece. **Treat the inside of the pieces with special care; avoid contact with gloves, dust and ash from the exterior.**
- If pieces containing a brownish interior are observed, remove a portion of the brownish material. The sample should be at least 2 cm³, with the maximum to be determined with reference to the available laboratory equipment.
  - If enough brownish material is available, collect at least two samples in separate reaction tubes/vials.
  - If insufficient brownish material is available, collect one brownish sample and collect at least one black sample (see below).
- If only pieces with a black interior are observed, collect a sample from the interior. The sample should be at least 2 cm³, with the maximum to be determined with reference to the available laboratory equipment. Collect at least two samples in separate reaction tubes/vials.
- If only small pieces are present, select at least two pieces. Remove dust/sand/potential contaminants with a sterile scalpel. Collect the ‘cleaned’ samples in separate reaction tubes/vials.

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5.2.2. SAMPLE CLEANING

OBJECTIVE

Remove exogenous (sources of) DNA, without destroying the remaining original DNA.

If samples originate from the interior of larger fragments of charcoal, and sampling has been performed in an adequate manner, this step should be omitted to preserve the original DNA.

RATIONALE

The surface/exterior charcoal can be contaminated with exogenous sources of DNA. The structures meant to protect the original DNA in wood can be damaged by the charring process, thereby leaving the remaining (degraded) original DNA more unprotected and fragile.

PROCEDURE

- Rinse the outside of samples in a 5% sodium hypochlorite (bleach) solution for 30 seconds, followed by a wash with molecular biology grade water. Do not ‘soak’ samples; the interior of charred wood is likely to absorb the solution, thereby destroying the remaining endogenous DNA.
5.2.3. DNA EXTRACTION AND CONCENTRATION

OBJECTIVE
Extract the available DNA in a small volume

RATIONALE
DNA can be extracted from charred wood using a variety of methods and commercially available kits, from silica spin column based kits to organic extractions. A suitable method/kit should:

- Have a high yield (retain as much DNA as possible)
- Remove components that may negatively influence further analyses (PCR inhibitors)
- Be assessed for quality and potential contamination through (negative) controls/reagent blanks and/or entry control prior to usage.

By ensuring the DNA is extracted in a small volume (or the extract is concentrated to a small volume), the amount of DNA that can be added to subsequent reactions is maximized.

PROCEDURE/CONSIDERATIONS

- Ensure that the number of controls in each extraction is sufficient. **Always include at least one reagent blank/negative control sample when working with charred wood samples.** Depending on the laboratory procedures and the reagents/kit that are used, a positive reagent control may be included in each test, each series of tests or per batch of reagents. Ensure that the sample used for this control is selected carefully: such a sample will contain more DNA than charred wood, causing it to become a potential source of contamination. It should therefore be easily recognizable in the unfortunate event of contamination; select a species that is not expected in charred wood investigations, with a very different DNA sequence.

- Grind the samples (including controls) until completely powdered. A ‘within tube’, ‘single use’ grinding system (such as bead beat solution, TissueLyser with 2 ml reaction tubes and stainless steel beads, FastPrep system with Lysing Matrix tubes or comparable) is preferred to sterile mortar and pestle, to minimize risk of contamination. **Complete grinding of charred wood is less demanding than grinding untreated wood.**

- Extract DNA using a suitable extraction method, optimized for high yield and allowing further concentration. E.G. 9, 10

  **Prolonged initial incubation should be considered.**

  Incubation with polyvinylloyrolidone (PVPP) (during initial incubation or after extraction) can be considered if inhibition is observed E.G. 11, 12. Ensure the (concentrated) elution buffer does not inhibit PCR.

  If silica columns are used, ensure elution is performed in pre-warmed elution buffer and sufficient incubation time (at higher temperature) is included. Consider use of carrier RNA/DNA. Simple reduction of elution volumes may negatively influence DNA yield if silica columns are used – consider the concentration of elution volumes instead 13.

- If the DNA is present in a volume larger than 10 µl, concentrate the DNA extract to 10 µl. A variety of methods can be used (commercial kits, filters, ethanol precipitation). If samples are concentrated, ensure the extraction blank is also concentrated. Ensure appropriate reagent controls are in place for concentration reagents (per batch).

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9 Lendvay et al., Improved recovery of ancient DNA from subfossil wood - application to the world’s oldest Late Glacial pine forest, Nyt phytologist (2017), doi: 10.1111/nph.14935
11 Rachmayanti et al., DNA from processed and unprocessed wood: Factors influencing the isolation success, Forensic Science International: Genetics, Volume 3 (3) (2009), https://doi.org/10.1016/j.fsigen.2009.01.002
13 Dilley et al., Methods for ensuring the highest DNA concentration and yield in future and retrospective trace DNA extracts, Science & Justice 61(2)193-197, https://doi.org/10.1016/j.scijus.2020.11.005
5.2.4. MARKER AMPLIFICATION AND SEQUENCING

OBJECTIVE
Amplify and sequence informative DNA marker(s)

RATIONALE
Due to the lack of high-quality DNA, large amplicons (>100 bp) are rarely obtained. A universal DNA marker for plant species identification with small amplicon size (<100 bp) is not available. Plant DNA markers that can be obtained with small amplicons, are generally either not informative enough to identify samples at species level, or can only be amplified in selected families. The forensic question – *what do laws or regulations require?* – dictates whether markers enabling family/genus level classification are sufficient or not.

Amplification of selected markers, confirmation of amplification and sequencing of confirmed amplicons may be performed with a variety of reagents, consumables and laboratory equipment.

PROCEDURE/CONSIDERATIONS

- Select the appropriate DNA marker to answer the forensic question at hand.

Appropriate markers may include:

- P6 loop – a portion of the *trnL* (UAA) intron, universal primers *trnL g/h* \(^14\) or adapted primer pair \(^15\): sufficient for at family/genus level identification, approximately 100 bp in Fabaceae.

- Mini-rbcL – a portion of rbcL, universal primers \(^16\), between 100 - 200 bp.

- A mini-barcode designed specifically to amplify and discriminate between genera/species within a (sub)family. Both *ycf1b* \(^17,18\) and *ndhF-rpl32* \(^19\) have been used for identification of (processed) wood samples of *Gleditsia* and *Pterocarpus* family Fabaceae, approximately 150-200 bp. Other/shorter mini-barcodes may be designed.

Selection of a new DNA marker or primer pairs should include a thorough study of inter- and intra-species variation in the amplified portion of the marker.

- Ensure that the number of controls in each PCR is sufficient. **Always include a PCR reagent blank/negative control sample and a positive PCR control sample when working with charred wood samples.** Ensure that the sample used for this positive control is selected carefully (see 5.2.3) and added in the correct concentration: if too much DNA is added, this can become a potential source of contamination. It should therefore be easily recognizable from samples and other control samples in the unfortunate event of contamination.

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\(^{17}\) Tan et al. The complete chloroplast genome of *Gleditsia sinensis* and *Gleditsia japonica*: genome organization, comparative analysis, and development of taxon specific DNA mini-barcodes. Sci Rep 10, 16309 (2020). https://doi.org/10.1038/s41598-020-73392-7


• Amplify the selected DNA marker in a small reaction volume (10-15 µl) with a high volume of concentrated DNA as template (approximately 5 µl).
Amplification of selected markers may be performed with a variety of reagents, consumables and machines.
Due to the use of small amplicons, (M13-) tailed primers are advised and 2 subsequent (or nested) rounds of PCR may be considered. Polymerases, reagents, pre-mixed mastermixes or additives eliminating PCR inhibition or otherwise maximizing PCR success are advisable. Reactions should be optimized prior to case work using relevant concentrations of DNA.

• Confirmation of amplification of the correct product (amplicon length) and absence of amplification products in all negative controls/reagent blanks must be confirmed by gel electrophoresis/bioanalyzer/comparable system prior to sequencing. Do not perform a sequencing reaction if no amplicon is observed.
The success rate of DNA amplification from charred wood is by no means 100%. A positive control sample should be assessed to determine whether an extraction/reaction may have been unsuccessful, or whether the result is inherent to the (sub)sample.
Contaminating DNA on samples or in the laboratory may lead to amplification of undesired products. If products are detected in reagent blanks/negative control samples, these may be sequenced to identify the source of contamination. Results from samples in the concerning reaction/test should be treated with extreme caution or be disregarded altogether.
If multiple products or products with an unexpected length are observed in samples (but not in controls) this may indicate the presence of contamination of subsamples. These may be sequenced but the results should be treated with extreme caution.

• Sequencing of confirmed amplicons may be performed with a variety of reagents, consumables and laboratory equipment (from sample purification and sequencing reaction to data collection platforms).
When Sanger sequencing, due to the use of small amplicons, bidirectional sequencing primers that can bind to the (M13-) PCR tails are advised. The use of primers synthesized with AMBeR chemistry or comparable may significantly improve read length.

5.2.5. ANALYSIS AND INTERPRETATION OF RESULTS

OBJECTIVE
Interpret sequence data to enable valid taxon identification (species/genus/family level)

RATIONALE
Analysis of sequence data of samples and controls is needed to identify potential errors and contamination issues, as amplification and sequencing of DNA from charred wood may more often result in partial sequences than when pristine DNA is used.

Manual review of sequences is required to eliminate incorrect/misleading basecalls and spacing errors, as even a single incorrectly called base or spacing error can leads to a significant drop in identity to reference sequences in such short sequences.

Comparison to private or public reference databases requires thought.

PROCEDURE/CONSIDERATIONS
• Manually review the automatically called sequence.
Manually check the electropherogram for abnormalities (spacing, mixed bases, low sequence quality) and first and last called positions of Sanger sequences as these can be less reliable. Heterogeneous positions/mixed bases (more than one base present) may indicate true heterogeneous sequences, contamination or sequence errors. Edit if necessary. This step can be performed with a variety of programs. Save/Export the reviewed sequence for analysis in the desired format (e.g. fasta).

• Compare (forward and reverse) sequences obtained from a single amplicon (using available in-house software or online tools). If full length, high quality sequences were obtained, these sequences should be indistinguishable and comparison will result in a single consensus sequence. If only partial sequences were obtained, a full-length sequence may be composed from the forward and reverse sequence.

If high quality sequences cannot be aligned, both sequences should be assessed separately. However, the results should be used for troubleshooting rather than for the case investigation.

• If consensus/composed sequences from both subsamples of a sample were obtained, compare these. If (near) identical sequences are obtained from both samples, only one sequence needs to be assessed; the sequence with the highest quality is most practical. If different sequences are obtained, both should be assessed separately (one sequence may represent foreign DNA, whilst the other may represent original DNA).

• Compare all relevant sequences to relevant reference sequences (such as each other, private databases or public databases) to identify which level of taxonomic identification is possible, and to which taxon the sequence belongs.

Before it is possible to ‘identify a sample’, it is necessary to determine which level of taxonomic identification is valid. This includes assessing if sufficient relevant sequences are available and if interspecies/genus/family variation is sufficient to exclude all other members of the genus/family/order (see also chapter 12.2 (i) in the ENFSI best practice manual21 and Figure 5.2.2).

Different databases come with different advantages and issues. Private databases rarely contain the diversity of sequences that public databases contain. However, public databases are seldom curated to the level that is expected from private databases. Before sequences from public databases can be trusted, some form of validation should be performed that may include answers to the following question: do the sequences ‘fit’ phylogenetically, have they been published, have different studies/research groups deposited (near) identical sequences.

Peer review of results such as base calling and/or taxonomic conclusion is good practice.

Figure 5.2.2. An example of assessment of the possible level of taxonomic identification of Senegalia mellifera KR738628.1 based on P6-loop sequence and NCBI GenBank.

5.2.6. REPORTING OF DNA BASED TAXON IDENTIFICATION

OBJECTIVE
Report a biologically valid, legally useful taxon identification

RATIONALE
In all cases, the reported identification should be biologically correct. The reader of the report should be supported to understand the results and conclusion of a report. Failing to provide such support may lead to misinterpretation of the results.

Depending on the legal system, legal requirements and which forensic question has been specified for the case at hand, specific requirements may have to be explicitly addressed in a report, whilst other data may be recorded and stored in a case file. If compliance with specific norms, guidelines, or standards is required, this may also impact what must be explicitly addressed in a report, or what may be recorded and stored in a case file.

PROCEDURE/CONSIDERATIONS

- Explicitly state that the identification was performed by analyses of (a) DNA marker(s).

- It may be desirable or required to also specify information about the laboratory procedures, the primers or, for example, the length of the sequences that were obtained and used for the eventual identification.

- Explicitly state which database/databases were used for identification. It may be desirable or required to specify information on the origin of (voucher) samples in private databases or the degree of curation of public databases.

- Explicitly state the taxonomic level of identification (e.g. species/genus/family) that is reached and the taxon name.
  For example: the sample is identified as species x, genus y or family z. Although it may be sufficient to state that the marker that is used is valid for identification at the genus (species/family) level, it may be desirable or required to specify information on the match % with reference sequences or the exclusion of nearest neighbors.

- If relevant: add other biological information that may not be evident for non-biologists. For example: a common name or the family the identified species belongs to. If it is illegal to import wood from a certain biological family, it may be of more value to conclude (or at least state) that the sample belongs to that specific family, than to conclude that the sample belongs to a specific species (leaving the non-biologist to decide whether the species is part of the family or not) (See Figure 5.2.3).

<table>
<thead>
<tr>
<th>QUESTION</th>
<th>POTENTIAL ANSWER</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the identity of the sample?</td>
<td>Genera Senegalia or Acacia (both tribe Acacieae, Family Fabaceae, synonym Leguminosae)</td>
</tr>
<tr>
<td>Is this sample Senegalia mellifera</td>
<td>This sample could originate from Senegalia mellifera, however several other species of Senegalia and Acacia cannot be excluded either.</td>
</tr>
<tr>
<td>Is this sample Acacia mearnsii?</td>
<td>No – this sample does not originate from Acacia mearnsii. (80-60% identity, published by 3 different groups)</td>
</tr>
<tr>
<td>Is this sample Leguminosae?</td>
<td>Yes – this sample originates from a species in the genus Senegalia or Acacia (both tribe Acacieae, Family Fabaceae, synonym for Leguminosae)</td>
</tr>
</tbody>
</table>

Figure 5.2.3. Examples of questions and corresponding answers, given the results & interpretation shown in Figure 5.2.2.
ANNEX

CHAIN OF CUSTODY TEMPLATE
### EVIDENCE TRANSFERRED

<table>
<thead>
<tr>
<th>AUTHORITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>EVIDENCE ITEM</th>
<th>EVIDENCE BAG NO.</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

### TRANSFER DETAILS

<table>
<thead>
<tr>
<th>RELEASED BY</th>
<th>RECEIVED BY</th>
<th>PACKAGING INTACT?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAME</td>
<td>DATE</td>
<td>NAME</td>
</tr>
<tr>
<td>SIGNATURE</td>
<td>TIME</td>
<td>SIGNATURE</td>
</tr>
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<td></td>
<td></td>
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<tr>
<td>NAME</td>
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<td>SIGNATURE</td>
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</tbody>
</table>
ANNEX

FORENSIC REPORT TEMPLATE
examples include:
- Analysis of charcoal confiscated in xxx on ddmmyyyy.
- Charcoal investigation of shipment seized on ddmmyyyy from vessel yyyy at harbor zzzz.
- Analysis of charcoal found in xxxx on ddmmyyyy.
- Classification of xxxx samples collected on ddmmyyyy.
## Case details

<table>
<thead>
<tr>
<th>Our reference</th>
<th>e.g. case registration number other relevant codes/numbers/names report number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of receipt</td>
<td>date of receipt of samples and/or questions</td>
</tr>
<tr>
<td>Your Reference</td>
<td>relevant number or code of commissioning party</td>
</tr>
<tr>
<td>Processing of items of evidence of this (sub) examination</td>
<td>Describe what will happen with the (remainder) of exhibits/samples</td>
</tr>
<tr>
<td>Annex(es)</td>
<td>-</td>
</tr>
<tr>
<td>General information Substantive information</td>
<td>Telephone number/ email address name of person responsible for issuing the report</td>
</tr>
</tbody>
</table>

**Examples include:**
- DNA extracts are stored at the laboratory name.
- Exhibits will be returned separately to xxxx
- Exhibits have been used completely for the analyses
1 Description of exhibits

Received from
person/organisation
Via
delivered in person/mail/…
Date of receipt
dd mm yyyy

Table 1 Description of exhibits

<table>
<thead>
<tr>
<th>Exhibit numbers</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>16ABC070619</td>
<td>Bag of charcoal, chosen randomly, from shipment on vessel xxx, seized at dd-mm-yyyy</td>
</tr>
<tr>
<td>02EVS210311</td>
<td>6 pieces of charcoal sampled from evidence item 1 (01EVS210311) on 21 march 2011 15:45, packaged in evidence bag with number BS00007308</td>
</tr>
<tr>
<td>Sample#5</td>
<td>Around 1 kg of largest chunks of charcoal sampled from a bag in the shipment seized on dd-mm-yyyy.</td>
</tr>
<tr>
<td>Charcoal05ABCDEMMYYYY</td>
<td>Handful of charcoal sampled from the 7th bag in the 3rd row on the top (6th) layer from the shipment on vessel xxx, seized at dd-mm-yyyy</td>
</tr>
</tbody>
</table>

Additional information: Noteworthy information, for example on packaging, storage conditions, chain of custody, etc.

2 Issues to be examined

As described in order form/email/court order/xxxx the following analysis is requested: 
“exact request/order from commissioning party”

examples include:
- What kind of charcoal is in the seized shipment?
- Is the charcoal in the seized shipment from Acacia trees?
- What material is in the seized shipment?

3 Information known to examiner

As described in order form/email/court order/xxxx the following relevant information was provided:

And/or

The following relevant information was provided by xxx during a meeting/telephone call/yyy at dd-mm-yyyy:
4 Examination

Depending on the amount of charcoal provided either the whole or a part of each sample is examined. If only a part of a sample was examined the selection of that part can be described in this chapter. For instance: The bag with number 16ABC070619 was opened and 8 pieces of charcoal were randomly selected.

The classification key in the guide 'Charcoal, guide for forensic classification' was used to classify the samples (or the X pieces of charcoal from sample Y) given in Table 1.

If not all the samples are suited for this guide, for instance because they’re not charcoal, this should be mentioned here. Other types of analyses applied to some or all of the samples, such as DNA or SEM, should be mentioned here as well.

5 Results

Any additional morphological descriptions can be added if relevant. For instance observations after opening the sample, such as the charcoal was damp and covered in mould, or all pieces of charcoal were smaller than 1 centimeter.

Based on the features of [feature 1], [feature 2] and [feature 3] sample X was classified as [result of key].
Based on the features of [feature 1], [feature 2] and [feature 3] sample Y was classified as [result of key].
Based on the features of [feature 1], [feature 2] and [feature 3] sample Z was classified as [result of key].

Alternatively:
Based on the features of [feature 1], [feature 2] and [feature 3] charcoal piece X from sample Y was classified as [result of key].

6 Interpretation of results

Of the [number of samples analysed] charcoal samples taken from shipment yyy, [number of classified samples] were classified as [result of key]. This means that [interpretation of table 2].

If some samples are classified differently than the rest (repeat for each category that is classified):
In addition, of the [number of samples analysed] charcoal samples taken from shipment yyy, [number of classified samples] were classified as [result of key]. This means that [interpretation of table 2].

If all samples could not be classified:
Of the [number of samples analysed] samples taken from shipment yyy, none could be classified. This means that [choose one of the relevant options of table 2 for zero classified samples].
7 Conclusion

Samples from the charcoal in the shipment xxx, seized at dd-mm-yyyy from vessel yyy, were analysed.
The charcoal in this shipment cannot be classified or
There is no charcoal in this shipment or
Part of the charcoal in the shipment consists of [classification] and/or
More than 50% of the charcoal in the shipment consists of [classification] (95% confidence level).

Signature

I declare to have drawn up this report truthfully, fully and to the best of my knowledge as expert xxxxxxxxxxxxxxxxxxxxxx.

Place xxxxx
Date dd mm yyyy

Reported by name of person responsible for issuing the report
IF YOU WOULD LIKE TO KNOW MORE ABOUT WHAT UNODC IS DOING TO SUPPORT STATES TO COMBAT MARITIME CRIME, PLEASE VISIT: HTTPS://WWW.UNODC.ORG/UNODC/EN/PIRACY/INDEX.HTML