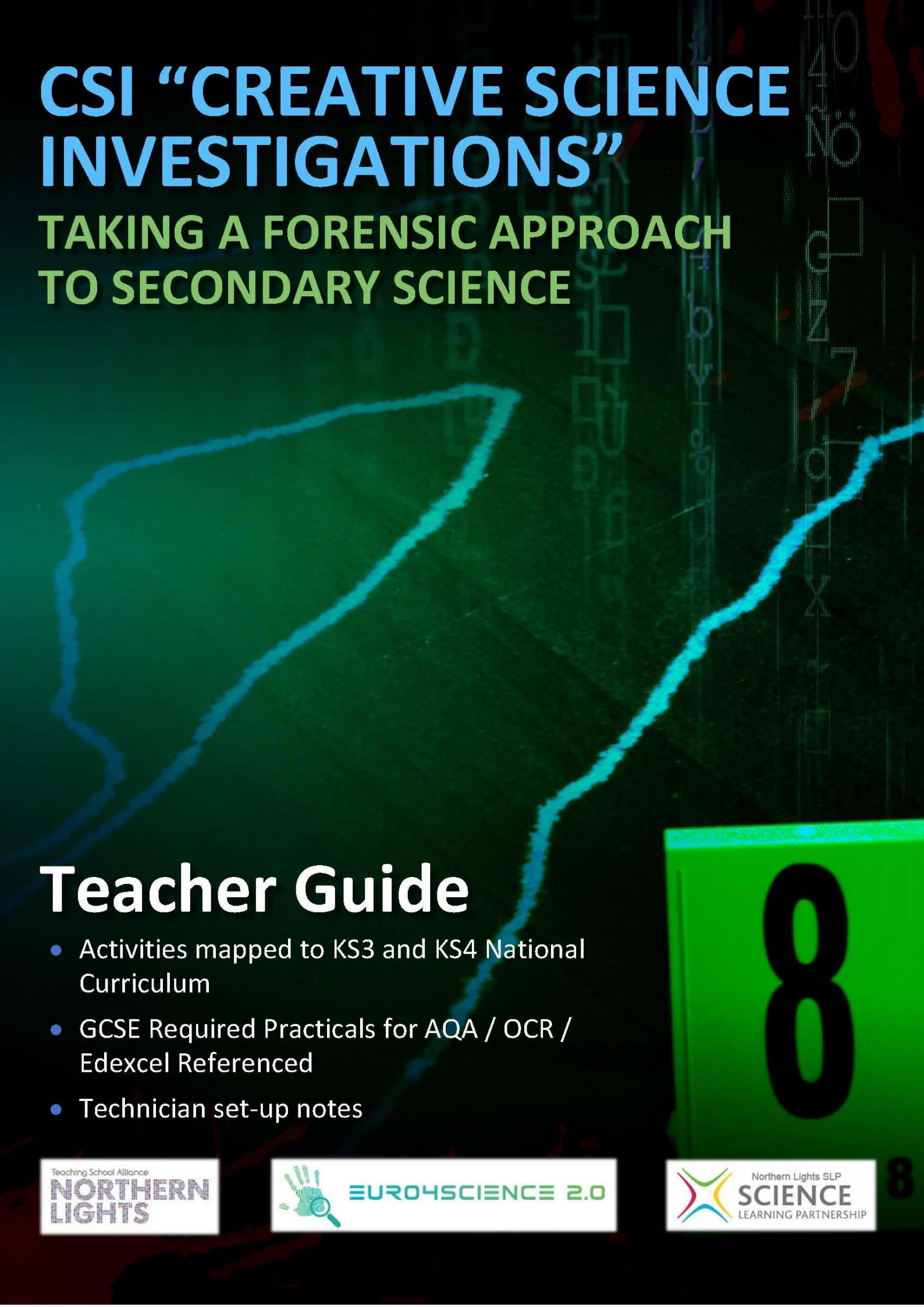
CSI "CREATIVE SCIENCE INVESTIGATIONS"

TAKING A FORENSIC APPROACH TO SECONDARY SCIENCE



Teacher Guide

- Activities mapped to KS3 and KS4 National Curriculum
- GCSE Required Practicals for AQA / OCR / Edexcel Referenced
- Technician set-up notes

Teaching School Alliance NORTHERN LIGHTS

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Northern Lights SLP SCIENCE LEARNING PARTNERSHIP

Contents

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HOW TO USE THIS GUIDE

This Toolbox Teacher Guide has been developed to provide information on how to implement the Forensic Science activities in the classroom. It has been reviewed and written in conjunction with UK Science Curriculum experts. Activities have been extensively trialled and reviewed to ensure they meet the advice and requirements set out by CLEAPSS. Links to Key Stage 3 and 4 "Working Scientifically" (WS) statements and with the GCSE Required Practicals for AQA, Edexcel and OCR are highlighted throughout, as are associated Apparatus and Techniques (ATs) skills covered. A summary of these are found on page 166.

This teacher guide contains four main chapters to be used with students. Although much of the content is best suited for Key Stage 4, there is much which can be used with younger years, as well as some material which teachers may adapt to enhance sixth form study. These chapters help students better understand the nature of the various forensic disciplines (blood analysis, fingerprints, botany, entomology, DNA analysis, toxicology, and general forensics) as they think critically about authentic situations. Students will learn to apply the skills used by forensic specialists to observe, recover, analyse, identify and explain evidence.

Each chapter includes:

- Learning Objectives
- Background context and scientific information for each topic
- Case studies, providing real-life examples of forensic science
- Weblinks to support further research
- Practical activities including technician notes
- Suggested answers to Student Guide questions

For each chapter, you will find the following symbols that associate the chapter to a subject of study, alongside the relevant WS and ATs.

This guide was developed to be used in conjunction with the Forensic Toolbox, which contains many of the materials needed for the practical activities. Additional resources should be found either within a well-stocked secondary science department, or readily available through normal school suppliers. Wherever possible, activities have been designed to minimise impact on science budgets whilst still providing activities and inspiration for engaging science using the theme of forensic science.

In order to help you apply this guide, three fictitious cases are presented that may be used in accordance with the different activities proposed – helping students solve a crime whilst learning about the science behind being a Crime Scene Investigator. Each activity can also be used on its own, making it easy to incorporate these into existing schemes of work.

We hope you enjoy using this resource within your classroom. We would welcome your feedback and comments – email slp-admin@sghs.org.uk

April 2019

CASE 1

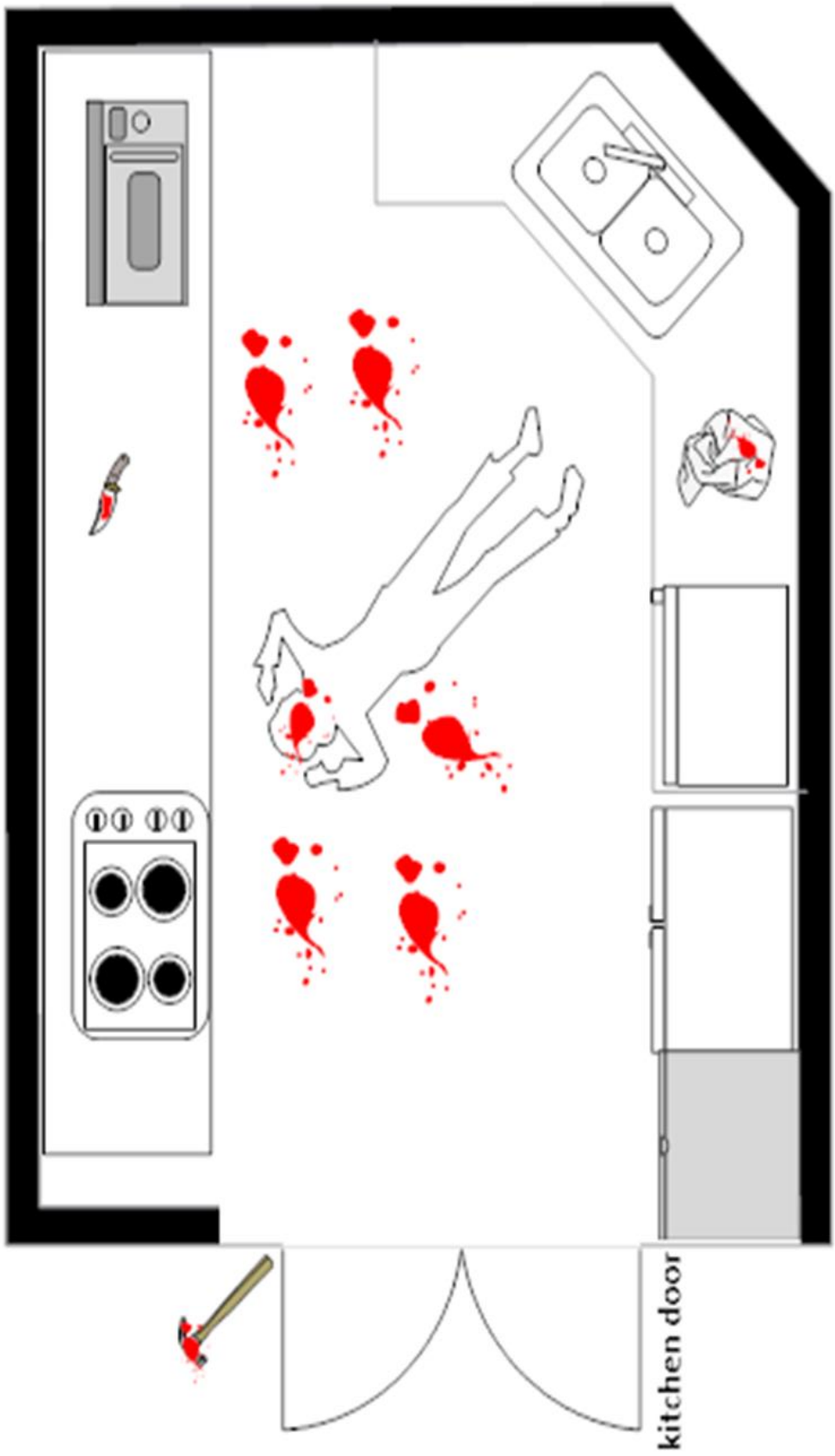
Early one morning, Tom Smith had gone to borrow his neighbour's lawnmower, as his had broken. He found the front door slightly open, which was unusual. He pushed the door open, calling for Josie Barrows, the elderly lady who lived there with her son, but there was no answer. About to leave, he noticed red spots on the kitchen door opposite. Alarmed, he ventured into the house and found the lifeless body of her son, a 27-year-old man, lying on the floor. Tom immediately left the scene and called the police.

The victim was named as Steve Barrows. His parents were divorced, and although Steve lived with his mother, he often saw his father, Charles Barrows, although the neighbours said that their relationship had never been good.

At the crime scene, the medical examiner found that the victim had sustained severe injuries to the head and determined the probable time of death was at 23h45. Around the body were several pools of blood and signs of fighting were verified – noted to be defensive injuries.

The CSI team labelled and packed various objects to send for analysis: samples of blood stains found on the floor (Evidence#1) and on the kitchen door (Evidence#2); a hammer that was behind the kitchen door (Evidence#3); a knife that was on the kitchen table (Evidence#4) and a towel found under the bench near the kitchen sink (Evidence#5). All objects had red spots. Fingerprints were found on the knife and hammer. In total, two different fingerprints were taken, which matched those found on nearby glassware. These glasses were removed for examination, and after processing them, it was determined that one matched the victim and the other did not.

All across the room, the CSI team found signs of disorder, pointing towards theft or the search for something particular. However, nothing was found to be missing. The suspects are: the father, whose relationship with his son was troubled (Suspect 1); the mother, who was being medicated for suffering from schizophrenia and who apparently did not take the medication regularly (Suspect 2); and a cousin, who had robbed the house more than once, but only stole things of reduced value (Suspect 3).


kitchen door

CASE 2

At 8:40am, following a particularly noisy University party, an unsuspecting cleaner found a 23-year-old student lying dead in the ladies toilets behind the bar.

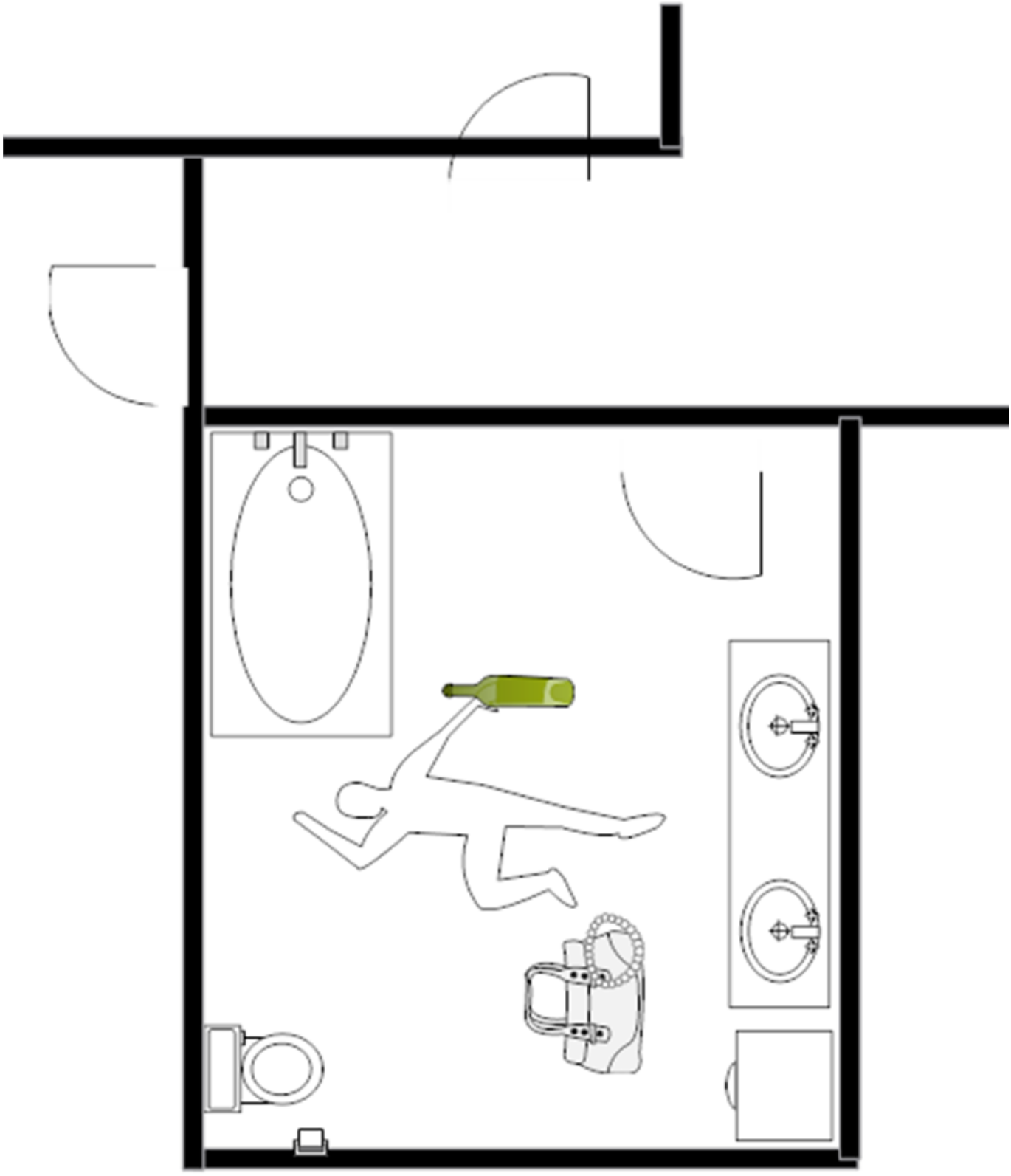
The victim was found face down and wearing the clothes from the night before. In her right hand, the victim had an empty whisky bottle. Beside the body was the victim's handbag.

When the body was examined, an emergency medical card stating that the victim was insulin dependent (diabetes type I) was found in her trouser pocket. In her handbag were blank sheets, some folded and others torn, with only her name on them (Evidence#6) together with a black pen (Evidence#7). Experts sent the sheets to the laboratory for further analysis and found that invisible ink had been used to send some threatening messages addressed to the victim, as well as fingerprints from the victim and another person.

In the autopsy room, the medical examiner took samples of the victim's liver (Evidence#8) to analyse the possible collateral effects of ingested alcohol. Since the victim was diabetic, toxicological blood tests (Evidence#9) were made to confirm this disease and to assess whether this was the cause of death.

During interrogation, the victim's colleagues admitted to the police that the last time they saw the victim alive she was arguing with her ex-boyfriend, who had a history of violence.

After all the interviews, the police had 2 main suspects. The first one was the victim's ex-boyfriend, who was found with a black pen (Evidence#10). The other suspect was a victim's friend whose strange behaviour during routine questioning had raised suspicions as to her own involvement with this case.



CASE 3

One early Sunday morning, a group of friends were walking in a forest when suddenly they smelled something strange. A little further on the source of the odour was revealed as they found a human cadaver. In order to not destroy the crime scene, the walkers didn't approach and immediately called the police.

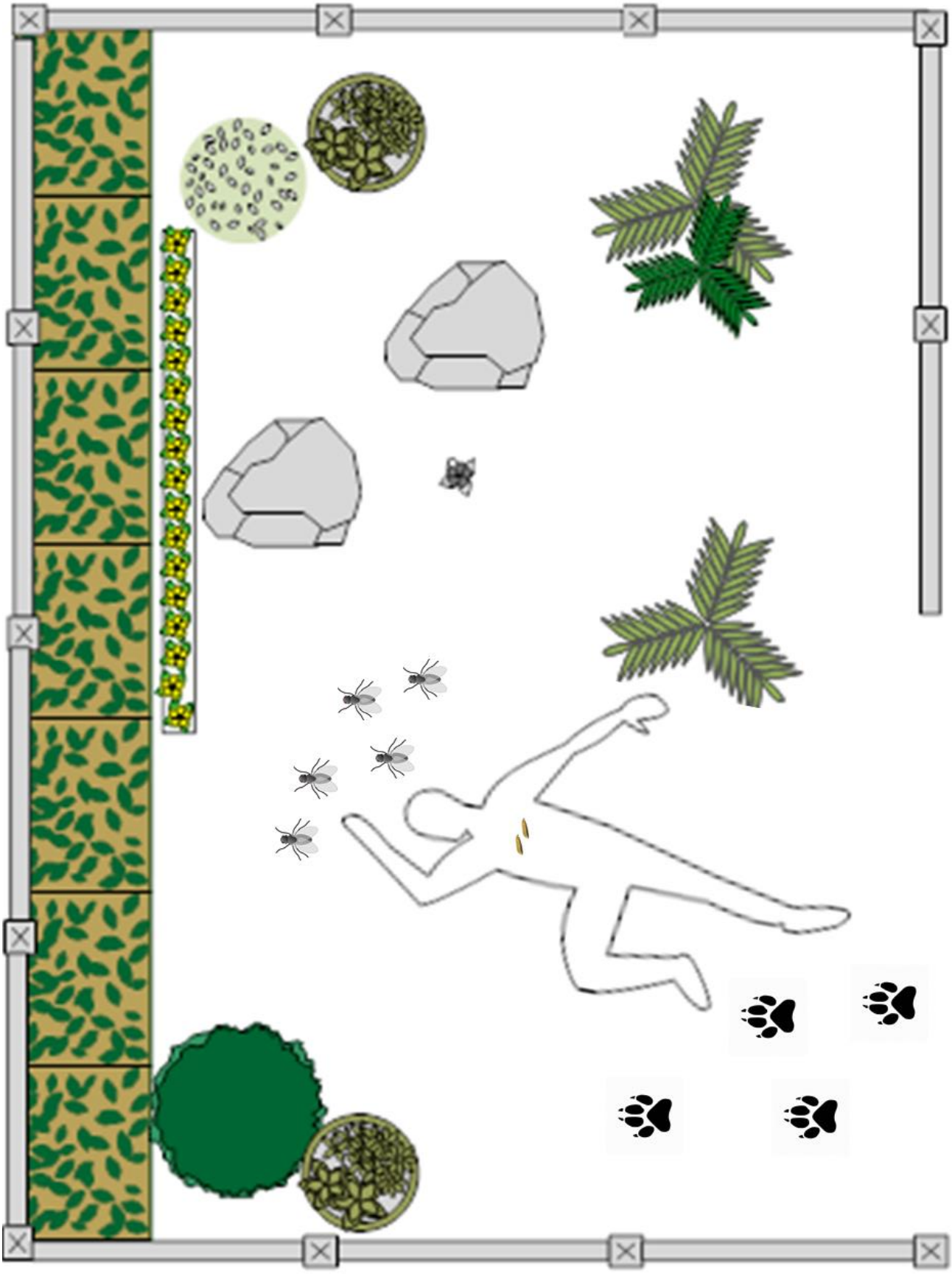
Since the crime scene was difficult to access, the CSI Team began to analyse the surrounding territory. At first sight, they found marks which suggested that where the body was located was not the original crime scene, but that it had been dragged there. Near to the body were footprints (Evidence #11) that, while not appearing human, could be relevant to the investigation.

Swarming around the decomposing body were several flies as well as insect larvae that were developing in the abdomen (Evidence#12). All of the insect traces were collected and taken to the laboratory to be analysed in order to help find the time of death.

The body was taken to the Legal Medicine Institute and the autopsy began with an external examination. The medical examiner discovered some hair which did not seem to belong to the victim (Evidence #13).

Inside the victim's nose were found pollen grains (Evidence#14) – a sample was sent to the laboratory of forensic palynology in order to determine their origin and hopefully point to the location of the original crime scene.

After all the forensic investigation, the CSI team concluded that the cadaver belonged to a 37 year old man who had been reported missing for about a week. How he died –why and where – was under further investigation.



Summary of Evidence, Suspects and Activities

Case Study 1: Who killed Tom Smith?

Evidence	Suspects	Activities
Evidence#1 – Blood stains on the floor	Suspect 1 – the father	Activity I – Is this really blood?
Evidence#2 – Blood stains on the kitchen door	Suspect 2 – the mother	Activity I – Is this really blood?
Evidence#3 – Fingerprints on hammer	Suspect 3 – the cousin	Activity V – Dusting and Lifting Latent Fingerprints Activity V – Dusting and Lifting Latent Fingerprints
Evidence#4 – Fingerprints and blood on knife		Activity II – Blood Typing Analysis Activity III – DNA Profiling Activity V – Dusting and Lifting Latent Fingerprints
Evidence#5 – Blood spots on towel		Activity I – Is this really blood? Activity II – Blood Typing Analysis

Case Study 2: Who killed the student at the bar?

Evidence	Suspects	Activities
Evidence#6 – Writing on sheets of paper	Suspect 1 – the ex-boyfriend	Activity VI – Revealing Latent Fingerprints using Iodine Fuming Activity XII – Invisible Ink Activity XIII – The Colour of Guilt
Evidence#7 – Black pen found in handbag	Suspect 2 – a friend	Activity XIII – The Colour of Guilt
Evidence#8 – Samples of the victim's liver		Activity X – Alcohol effect on lived
Evidence#9 – Toxicological blood tests		Actvity XI – Sugar in Blood
Evidence#10 – Black pen found on ex-boyfriend		Activity XIII – The Colour of Guilt

Case Study 3: The corpse – how and where did they die?

Evidence	Activities
Evidence#11 – Footprints – not human	Activity IX – Hair and Footprints
Evidence#12 – Insect larvae from abdomen	Activity VIII - The Insect Clock
Evidence#13 – Hair taken from clothing	Activity IX – Hair and Footprints
Evidence#14 – Pollen grains from victim's nose	Activity VII - The Hidden Secrets of Pollen grains

INTRODUCTION

Forensic Science – *What is it?*

Forensic Science (often abbreviated to Forensics) is the practical application of scientific methods and tools to investigate crimes and bring suspects to trial. The term “Forensic” comes from the Latin word *forensus* meaning “of the forum”. In ancient Rome, the forum was the courthouse, where people debated matters of law in a public meeting place.

Real-life criminal investigation is almost never as fast and easy as it looks on TV shows and movies. On television, deoxyribonucleic acid (DNA) tests are completed almost instantly, but in reality they can take days, weeks, or even months. The investigators who work in crime labs, examining evidence from crime scenes, do not always come up with the “perfect match” to a fingerprint or a strand of hair.

Did You Know?

The world’s first forensic laboratory was the creation of Edmond Locard, one of the pioneers of forensic science. Locard became known as the "Sherlock Holmes of France".

History of Forensic Science

Fascination with forensics explains the popularity of many TV shows, movies, and books, but crime and science have been linked for a long time.

Fingerprints

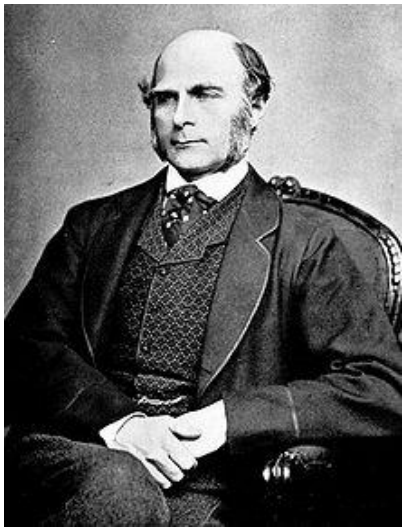


Figure 2 - Sir Francis Galton, considered the "Father of Fingerprinting".

The history of forensic science dates back thousands of years and one of the first sciences used was fingerprinting. The first application of fingerprints was in ancient Babylon (Pre-700 BC) where these were used on clay tablets for business transactions [Figure 1]. In 1686, Marcello Malpighi noted for the first time the ridges, spirals and loops in fingerprints.

Figure 1 - Ancient seal with a fingerprint.

However, fingerprints weren't used as a method for identifying criminals until the 19th century. In 1892, Sir Francis Galton published the book "Finger Prints", establishing the individuality of fingerprints and a first classification system. In 1896**,** Sir Edward Richard Henry, Commissioner of the Metropolitan Police of London, developed the fingerprint classification system (based on the direction, flow, pattern and other characteristics in fingerprints) that would later be used in Europe and North America. In 1910, Edmond Locard set up the first Forensic Lab in Lyons, France, and formulated the "*Locard´s* Exchange Principle": the theory that when a criminal comes in contact with an object or person, a cross-transfer of evidence occurs and the criminal either removes something from the crime scene or leaves something behind. Eight years later, in 1918, Edmond Locard suggested 12 matching points as positive fingerprint identification. In 1977, the FBI introduced the beginnings of Automated Fingerprint Identification System (AFIS) with the first computerised scans of fingerprints.

Entomology

Another old field of the Forensic Science is Entomology. The first documented forensic entomology case was reported by the Chinese lawyer and death investigator Sung Tz'u in 1235. He describes the case of a stabbing using a sickle near a rice field. All those in the village who owned a sickle were ordered to bring

them out and lay them in the sun. Flies gathered on one particular sickle, identifying it as the murder weapon. Faced with this scene, the man who had committed the crime confessed.

Toxicology

Until the 1700s, convictions associated with homicidal poisoning were based only on circumstantial evidence rather than the identification of the actual toxicant within the victim. In 1781, Joseph Plenic stated that the detection and identification of the poison in the organs of the deceased was the only true sign of poisoning. Years later, in 1814, Mathieu Orfila (considered the "Father of Toxicology"), published the first complete work on the detection of poisons and legal medicine. Another breakthrough was in 1836, when the English chemist James Marsh discovered an accurate way to detect arsenic in the body, known as the Marsh Test, and was the first to use toxicology in a jury trial.

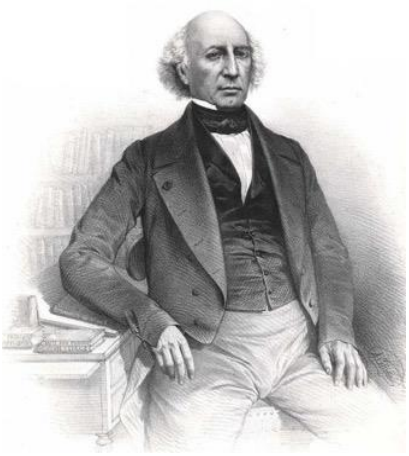


Figure 3 - Mathieu Joseph Bonaventure Orfila.

Haematology

Human blood also became part of Forensic Science, when Karl Landsteiner, in 1901, discovered that human blood could be grouped into different categories (A, B, AB and O). With his discovery of the blood groups and the development of the ABO system, Landsteiner won the Nobel Prize. In 1915, Leone Lattes published a work that illustrated the forensic value of the then new technique for ABO typing bloodstains. Although published 15 years after Landsteiner first described the ABO blood groups system in humans, this work is the first report of ABO typing of dried blood for forensic purpose.

Figure 4 - Karl Landsteiner, the "Father of Immunology".

Even before the discovery of the ABO system, the German scientist Schönbein, in 1863, first discovered the ability of haemoglobin to oxidise

hydrogen peroxide. This results in the first presumptive test for blood. In 1937, Walter Specht developed the chemiluminescent reagent Luminol as a presumptive test for blood.

Ballistics

Forensic Ballistics is another important area of Forensic Science. Henry Goddard was the first person to use physical analysis to connect a bullet to the murder weapon in 1835. The comparison was based on a visible flaw in the bullet, traced back to a mould. Bullet examination became more precise in the 1920s, when the physician Calvin Goddard created the comparison microscope to help determine which bullets came from which shell casings. In the 1970s, a team of scientists at the Aerospace Corporation in California developed a method for detecting gunshot residue using scanning electron microscopes. Recently an automated imaging system was developed called the Integrated Ballistics Identification System for comparison of the marks left on fired bullets, cartridge cases and shell casings.

Figure 5 – Coronel Calvin Hooker Goddard with Comparison Microscope.

DNA Testing

The identification of the structure of DNA by James Watson and Francis Crick in 1953, brought a tremendous change in criminal justice.

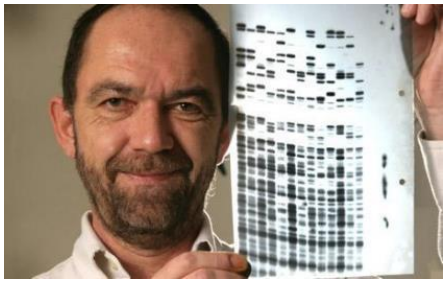


Figure 6 - Professor Sir Alec John Jeffreys, a British geneticist.

DNA profiling, as we know it today, was developed thanks to two independent breakthroughs in molecular biology that occurred at the same time. In 1983, Kary Mullis discovered the polymerase chain reaction (PCR) and in 1985, Sir Alec Jeffreys discovered a method of identifying individuals from DNA, called DNA fingerprinting. DNA profiling was originally developed as a method of determining paternity, to determine whether two

individuals have a biological parent–child relationship. In 1986, the England police asked Alec Jeffreys, who had begun investigating the use of DNA for forensics, to use DNA to verify the confession of a 17-year-old boy in two rape-murders in the English Midlands. The tests proved the teenager was in fact not the perpetrator and the actual attacker was eventually caught. In 1995, the world's first national DNA database began operations in the UK. Since the advent of DNA testing in 1985, biological material (skin, hair, blood and other bodily fluids) has emerged as the most reliable physical evidence at a crime scene.

Areas of Forensic Science

Forensic science is multidisciplinary, including a wide range of subspecialties that are used in the traditional sciences. Forensic Science must be understood as the set of all scientific and technical knowledge that are used in legal questions of criminal or civil nature. The most common forensic science areas are: Biology, Toxicology, Chemistry, Pathology, Fingerprints and Ballistics. However, there are other areas of expertise that can complement investigations, such as Anthropology, Odontology, Entomology, Botany, Computer Forensics, Bloodstain pattern analysis, Psychology and Document Analysis. In Table 1, there is a brief description of some forensic areas.

Table 1 - Fields of the Forensic Science.

Areas	Description
Forensic Anthropology	The application of physical anthropology in a legal setting, usually for the recovery and identification of skeletonised human remains.
Forensic Odontology	Study of the uniqueness of dentition, better known as the study of teeth. Forensic odontologists can identify human remains that cannot be identified using other methods, identify bodies from plane crashes, determine the source of bite mark injuries and estimate the age of skeletal remains.
Forensic Entomology	The examination of insects in, on and around human remains, to assist in the determination of time or location of death.
Forensic Pathology	Focuses on determining the cause of death by examining a cadaver or an injury in the context of a legal inquiry.

Forensic Biology	The application of biological analysis methods, particularly DNA analysis, to legal investigations. DNA analysis involves the investigation of bodily fluids that can be found at a crime scene, particularly blood, semen, saliva.
Forensic Botany	The study of plants and plant remains in the context of a criminal investigation. This area includes the analysis of wood, fruit, seed, branches, leaves, plant hairs, pollen, spores, and algae cells.
Computer Forensics	Also known as digital forensics, includes the recovery and investigation of material found on digital devices, with the purpose of identifying, preserving, recovering, analysing and presenting facts about the digital information.
Bloodstain pattern analysis	The analysis of bloodstains can provide vital clues as to the occurrence of events. The successful interpretation of bloodstain patterns may provide clues as to the nature of the crime, the possible sequence of events, any disturbance to the scene that may have occurred, and even the position of individuals and objects during the incident.
Forensic chemistry	The application of chemistry to law enforcement. Many different analytical methods may be used to reveal what chemical changes occurred during an incident, and so help reconstruct the sequence of events.
Forensic Psychology	The intersection between psychology and the justice system. It involves understanding fundamental legal principles, particularly the witness testimony.
Ballistics	The science of mechanics that deals with the launching, flight, behaviour, and effects of projectiles, especially bullets, gravity bombs or rockets.
Fingerprints	The detailed study of the impression left by the friction ridges of a human finger.
Forensic toxicology	A set of multiple areas beyond toxicology (such as analytical chemistry, pharmacology and clinical chemistry) to aid medical or legal investigation of death, for example in cases of poisoning and drug abuse.
Documents Analysis	The study of handwriting, typewriting, imprinted documents, alterations, ink, paper, and writing instruments. The primary aim is to gain as much information regarding the document as possible without damaging or altering the document if possible.

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Web sites

- "A simplified guide to forensic science", http://www.forensicsciencesimplified.org/
- "High School/College – Forensic Science", http://www.terrificscience.org/freebies/lessonexchange/forensics/
- "Explore Forensics", http://www.exploreforensics.co.uk/

CHAPTER 1:

HUMAN INDIVIDUAL IDENTIFICATION

Blood Analysis

Objectives

Students will be able to:

- Describe the composition of blood
- Explain the functions of blood cells
- Describe how to detect for the presence of blood
- Describe how to determine the blood type of a sample

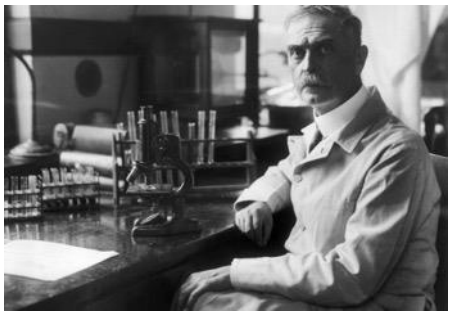


Figure 7 - Karl Landsteiner.

Blood left at a crime scene can be analysed in several ways by a criminal investigator.

With the discovery of the ABO system by Landsteiner in 1901, knowledge in human blood identification has expanded significantly. Since more than one person has the same blood type, blood typing may provide evidence that can be linked with a group of people, but not a specific individual. With the introduction of DNA typing technologies in the 1980s, and as white blood cells contain DNA, an individual bloodstain can now be identified through genetic variation at a molecular level.

Bloodstains often constitute the major physical evidence in crime investigation and are frequently found at different sorts of crime scenes, for example, homicide, hit-and-run, assault, robbery and burglary. In examining blood evidence, the questions which the forensic scientist must answer are: Is it blood? Is it human? Whose blood is it?

Composition of Human Blood

Blood is a complex fluid tissue and consists of two main components: plasma and formed elements [Figure 8]. Plasma is similar to salt water in composition, with a mixture of dissolved proteins, salts and other chemicals. There are three main types of formed elements (also called blood cells) that perform different functions: red blood cells (erythrocytes) that carry respiratory gases, mainly oxygen and carbon dioxide; white blood cells (leukocytes) that fight infection, remove dead/dying cells and destroy cancer cells; and platelets (thrombocytes) that aid in blood clotting and are involved in repairing damaged blood vessels.

Figure 8 - Composition of human blood.

Blood Typing

Blood typing is the classification of blood based on the presence or absence of antigenic substances on the surface of red blood cells (erythrocytes). Before DNA testing, blood typing was used as a method to match or exclude a certain suspect from a crime scene, by examining antigen-antibody reactions.

The ABO blood type classification system uses the presence or absence of the A and B antigens to categorize blood essentially into four groups: A, B, AB and O. [Table 2] . Blood can be further categorised according to the presence or absence of the Rh protein. Each blood type is either Rh positive (Rh+) if it has the Rhesus antigens, or Rh negative (Rh-) if it doesn't have the antigens. The study of these two characteristics enables more precise blood identification.

Did You Know?

The rarest blood type is AB (Rh -) About 85% of the population, have the Rhesus antigen.

Table 2 - ABO blood groups

Group	Antigens Present	Antibodies Present
Group A	Only the A antigen on red cells	B antibody in the plasma
Group B	Only the B antigen on red cells	A antibody in the plasma
Group AB	Both A and B antigens on red cells	Neither A nor B antibody in the plasma
Group O	Neither A nor B antigens on red cells	Both A and B antibody are in the plasma

Blood type tests are used to identify the type of blood, for example before a person gets a blood transfusion, to check a pregnant woman's blood type, to see if two people are likely to be blood relatives or to determine the blood type of a specimen found at a crime scene.

In a crime scene, if the blood type of a suspect is different from the one detected at the scene, this person probably did not commit the crime. But if a suspect's blood type is the same as the one found at the scene, then this person may be the criminal. At that point, only a DNA test can confirm whether any of the remaining suspects are the actual criminal.

Forensic Analysis of Blood

Presumptive Tests

In a large crime scene area and sometimes on a "cleaned up" surface, it may not be immediately obvious where to begin looking for hidden bloodstains and occasionally it is difficult to see them with the naked eye. In these cases, it is necessary to use chemical tests to reveal their presence.

In forensic laboratories there are two main types of tests to identify blood: Presumptive tests and Confirmatory tests. The Presumptive blood tests indicate the possibility of blood, but only confirmatory tests allow for the conclusion that blood is present. Presumptive blood tests, such as Luminol and Kastle-Meyer, are usually based on the colour change or chemiluminescence of a particular reagent when it comes into contact with the haemoglobin in blood. The confirmatory tests are necessary for the possibility of false positives with the

presumptive tests and to determine the species origin based on antigen/antibody interactions.

Luminol

Luminol is a chemical which, under certain conditions, gives off a blue glow when mixed with an appropriate oxidizing agent, such as hydrogen peroxide. Luminol is used as a presumptive test to detect trace amounts of blood left at crime scenes, as it reacts with iron found in haemoglobin.

Luminol is highly sensitive to the presence of small traces of blood, yielding positive results at dilutions as high as 100 000 000:1. However, this high sensitivity is accompanied by low selectivity. Many materials such as laundry bleach, many food items, iron and other metals produce false positive reactions that are indistinguishable from positive results caused by actual blood.

Luminol, at least in aqueous solution, is considered non-destructive and does not interfere with subsequent DNA analysis.

Did You Know?

In a 1937 paper, the German chemist Walter Specht was the first to suggest the use of Luminol as a presumptive test for forensic blood detection.

Kastle-Meyer Test

The Kastle–Meyer or phenolphthalein test is another presumptive blood test, which uses an alkaline phenolphthalein solution to detect the possible presence of haemoglobin. The Kastle-Meyer test is a catalytic colour test that will produce a bright pink colour, when phenolphthalein and hydrogen peroxide react with the iron molecules in haemoglobin.

This test is non-destructive to the sample, which can be further used in laboratory tests, since a small amount of blood sample is collected with a swab.

Did You Know?

The Kastle-Meyer test was introduced in 1901 by Kastle and improved in 1903 by Meyer.

Fingerprints

Biology Chemistry

Objectives

Students will be able to:

- Describe the characteristics of fingerprints
- Identify the basic types of fingerprints
- Explain how fingerprint evidence is collected
- Determine if a fingerprint matches a fingerprint on record
- Use the process of lifting a latent print

Fingerprints were a major breakthrough in forensic science, giving law enforcement around the world a new tool to help in crime investigation.

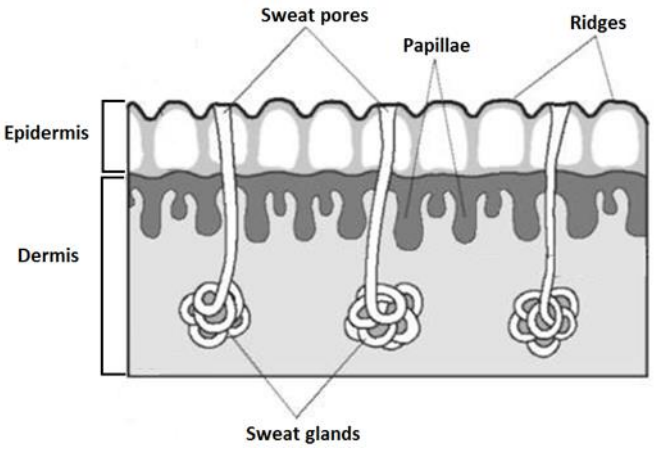


Figure 9 - Schematic representation of human skin layers and friction ridges.

A fingerprint, which is unique to an individual, is usually defined as an impression left by the dermal (or friction) ridges. Friction ridges are raised portions of the epidermis, located on the palms of the hands and the soles of the feet, [Figure 9]. These ridges are small extensions of the dermis into the epidermis, which help us grip the objects that we touch.

The imprint of fingerprints consists of natural secretions of sweat from the eccrine glands that are present in the friction ridge of the skin. These secretions are mainly a combination of water, oils and salts, but dirt from everyday activities is also mixed into these secretions.

Characteristics of Fingerprints

Fingerprints are named for their general visual appearance and patterns. These are called arches, whorls, and loops. Arches, the simplest fingerprint pattern (only 5% of the total population have arches) are characterised by ridge lines that enter from one side of the fingerprint and exit from the other side with a rise in the centre. Whorls look like a bull's-eye with two deltas (30% of the total population have whorls). Loops are characterised by ridge lines that enter from one side, either the right or the left, of the pattern, curve around, and exit from the same side they enter (about 65% of the total population have loops) [Figure 10].

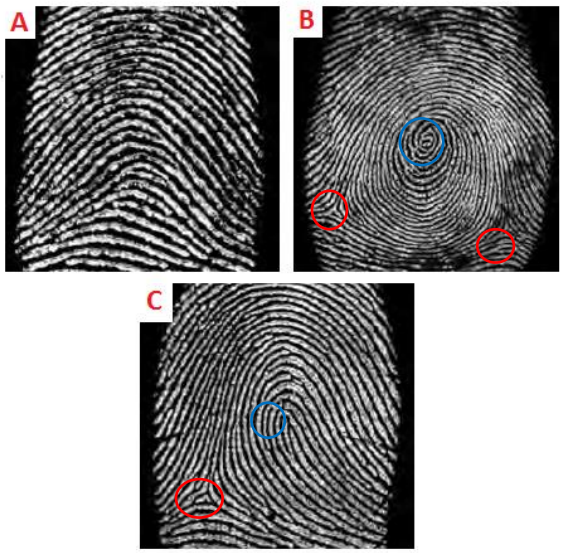


Figure 10 - Fingerprints patterns. A: Arches; B: Whorls and C: Loops.
Red circles – Delta; Blue circles – Core.

When forensic examiners look for a fingerprint, they see two things: the presence of a core and deltas. The core is the centre of a loop or whorl, and the delta is a triangular region located near a loop.

Types of Fingerprints

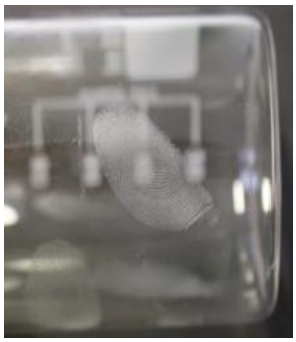
Fingerprints can be of three types: patent, plastic or latent. Patent fingerprints, or visible prints, are left on a smooth surface when blood, ink, or some other liquid comes in contact with the hands and is then transferred to that surface. Plastic fingerprints are indentations left in any soft material, such as clay, putty or wax, and are also visible. Latent fingerprints, or hidden prints, are caused by the transfer of oils and other body secretions onto a surface and may require treatment to be seen.

The detection process of latent fingerprints can be complex and usually requires the use of powder or a chemical reagent to produce a high degree of visual contrast between the ridge patterns and the surface on which a fingerprint has been deposited. Visual examination is always the first step in revealing latent

fingerprints, by using strong lighting that is completely non-destructive. After the visual examination, other methods may be used to reveal the fingerprints. Fingerprint powders, iodine fuming, and silver nitrate are considered the "classic" methods as they have been used since the 19th century. However, there are other methods frequently used such as superglue fuming.

One of the most common methods for discovering and collecting latent fingerprints is with fingerprint powders (black granular, aluminium flake, black magnetic, etc.), which are mainly used for dusting nonporous surfaces such as glass and polished metal. This is most commonly used to reveal latent fingerprints on immovable objects at crime scenes.

Since its discovery in 1976, superglue fuming, also called cyanoacrylate fuming from the primary component of superglue, became one of the most frequently used latent print development processes. This process is also used to develop latent fingerprints on nonporous glossy surfaces such as glass, plastic, and polished metal.



Did You Know?

Superglue fuming was discovered by accident in 1976 when Masao Soba notice white fingerprints on the surface of a super glue container. In 1980, Frank Kendall improved the process and adapted it to latent fingerprints.

DNA Profiling

Biology Chemistry Physics

Objectives

Students will be able to:

- Describe what DNA is
- Explain how DNA evidence is compared for matching
- Explain how to use DNA fingerprinting to identify DNA from a parent, child, or relative of another person

Did You Know?

99.9% of our DNA sequence is the same as other humans.

No two people on earth have the same DNA, with the exception of identical twins. In the past 20 years, DNA analysis has grown from a relatively minor forensic speciality to become a vital part of the work of any forensic lab. Since the appearance of DNA profiling in the 1980s, DNA has been used to investigate crimes, establish paternity and identify victims of war and large-scale disaster. Because each human is unique, DNA evidence from a crime scene or from an unidentified body can be traced back to a crime or eliminate a suspect.

There are several types of biological evidence commonly used in forensic science for the purpose of DNA analysis, such as: blood, saliva, semen, skin, urine and hair.

DNA Structure and Function

To understand how DNA is analysed in forensic science, it is important to know about the structure and function of DNA. DNA is a molecule that consists of two nucleotide strands held together by hydrogen bonds in a helical shape. The nucleotide molecule consists of a triphosphate group, a deoxyribose sugar and one of four nitrogenous bases (adenine, guanine, thymine and cytosine).

Did You Know?

If you unwrap the entire DNA you have in all of your cells, you could reach the moon 6000 times.

DNA is essentially the molecule that holds all of the information and instructions needed for an organism. An important propriety of DNA is that it can replicate (i.e. make copies of itself).

Genetic information is stored in molecules of DNA making up structures called chromosomes. The human genome is composed of over 3 billion base pairs of information organised into 23 pairs (a total of 46) of chromosomes in the nucleus of most human bodies. One chromosome in each pair is inherited from the mother and the other chromosome is inherited from the father. DNA in the chromosomes is called nuclear DNA and is virtually identical in all cells of the human body. Another type of DNA is found in the mitochondria of the cell. Mitochondrial DNA exists in the form of a circular loop and, unlike nuclear DNA, is passed to the next generation by the mother. Therefore, an individual's mitochondrial DNA is the same as their mother's mitochondrial DNA. Mitochondrial DNA is used forensically for several reasons since it exists in greater quantities than nuclear DNA.

Forensic DNA Profiling

Forensic DNA profiling, also known as DNA fingerprinting, is a technique employed by forensic scientists to identify individuals using the characteristics of their DNA.

Several steps are necessary before DNA samples can be analysed and compared. The first step in preparing a sample from DNA fingerprinting is to extract the DNA from the cell nucleus. The cells are isolated from tissue and are

then disrupted to release the DNA from the nuclear and cell membrane as well as from proteins and other cell components. The second step is the amplification of the DNA using a polymerase chain reaction (PCR), which amplifies certain pieces of DNA. The third and final step is electrophoresis.

Electrophoresis is the method of separating the molecules under the influence of an electrical field based on the size of the DNA fragments.

One of the most known electrophoresis techniques is gel electrophoresis. Gel electrophoresis is a porous matrix that is used to separate DNA molecules. The type of matrix used (the most common are agarose or polyacrylamide) depends on the size of the DNA fragments that will be visualized. Due to the difference in pore size of such matrices, agarose gel is usually used to separate fragments ranging from 0.2 kb to 50 kb (1 kb = 1000 base pairs) and polyacrylamide gel to separate small fragments up to 1kb.

Once the DNA is negatively charged (each nucleotide has a negatively charged phosphate attached to it), it will move toward the positive electrode under the influence of an electrical field. Larger molecules move through the gel more slowly, while smaller ones can slip through the pores faster. Thus, the fragments will be arranged according to the size. As the DNA migrates, the different fragments will form bands, which are composed of many identical copies of a particular-size portion of DNA [Figure 11].

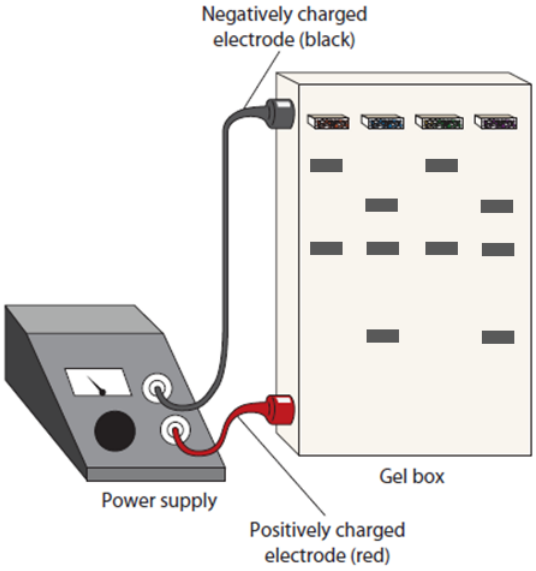


Figure 11 - Diagram of an electrophoresis apparatus.

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Real Life Case Studies

Stratton Brothers (1905)

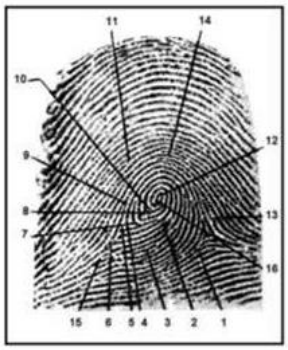


Figure 12 – Fingerprint analysis.

On the morning of 27th March 1905 in Deptford, England, 16-year-old William Jones visited the paint shop of Thomas Farrow and his wife Ann, but found the shop closed. Jones knocked on the door several times and, when he received no response, looked through the window. He was alarmed by the sight of numerous chairs knocked over, so he went for help. He approached a local resident, Louis Kidman, and the two forced their way into the shop around the back of the building. Once inside, they discovered the beaten dead body of Mr. Farrow in a pool of blood and the unconscious body of his wife.

Mrs. Farrow was rushed to hospital and the police were called. Unfortunately, she died a few days later. There were no signs of forced entry, however, an empty cash box was found on the floor, suggesting that robbery was the motive for the crime. The cash box was examined, and a greasy fingerprint was found on the inside that did not match the victims or any of the file of criminal prints that Scotland Yard possessed.

With the fingerprint evidence trail cold, the police began interviewing possible crime witnesses. Fortunately, a local milkman reported seeing two young men in the neighbourhood of the Farrow house on the day of the murders. Soon identified as brothers Alfred and Albert Stratton, the police began interviewing their friends. Alfred's girlfriend told the police that he had given away his coat on that day and changed the colour of his shoes the day after the murders. A week later, authorities finally caught up with the Stratton brothers and their fingerprints were taken. Alfred's right thumb was a perfect match for the print on the Farrow's cash box.

Fingerprint evidence became the prosecution's only solid evidence when the milkman was unable to positively identify the Stratton brothers. The court heard how fingerprinting worked as a reliable means of identification and how the print matched the accused perfectly. As a result, the Stratton brothers were convicted and hanged on May 23, 1905.

Ludwig Tessnow (1901)

Figure 13 - Ludwig Tessnow

In July of 1901, two brothers Herman and Peter Stubbe (6 and 8 years old) went out to play in the woods in Ruden, Germany, and they never came back. A search the next day found dismembered body parts of the boys dispersed through a wide woodland area.

A man named Ludwig Tessnow, a local carpenter, had been seen talking to the boys on the day they disappeared. A subsequent search of his home found freshly laundered clothes with suspicious stains that Tessnow said were wood dyes used in his carpentry work.

Three year earlier, in a different area of northern Germany, two young girls had been murdered in a similar way. Ludwig Tessnow had been detained for questioning in that murder as well and claimed at the time that the stains on his clothes were wood dyes.

Despite their suspicions, no hard evidence was available. Then they heard about a young biologist, Paul Uhlenhuth, who had developed a test that could be used to distinguish blood from other substances, as well as human blood from animal blood. Uhlenhuth examined the boots and clothing belonging to Tessnow and concluded that the clothing did contain wood dye as Tessnow has claimed, but also 17 spots of human blood and several stains of sheep's blood. Based on this evidence, Tessnow was found guilty and executed at Griefswald Prison.

Tommie Lee Andrews (1986)

Figure 14 - Tommie Lee Andrews

In May of 1986, a man entered the Orlando apartment of Nancy Hodge and raped her at knifepoint. After grabbing her purse, he left. During the succeeding months, he raped more women, making sure they didn't see his face, and on his way out he always took something that belonged to them. In six months, he had raped more than 23 women. However, he had made one mistake: he left behind two fingerprints on a window screen. When another woman eventually identified him as a thief, his prints were matched to those from the window screen and they had their man: Tommie Lee Andrews.

Although his blood group matched semen samples taken from several of the victims, and the single victim who had caught a glimpse of him had made a positive identification, proving him to be a serial rapist would be difficult. Therefore, the Florida DA decided to try DNA technology and concluded that blood and semen sample were identical. This was the first case to introduce DNA typing into a US court.

Tommie Lee Andrews was detained and linked to the rapes by conventional fingerprint and DNA profile evidence. He was sentenced to more than 100 years in prison.

ACTIVITIES

Activity I: Is this Really Blood?

Objective:
Use the Kastle-Meyer presumptive blood test to determine if a given stain contains blood.

Time required:
Kastle-Meyer Test – 15 minutes

Curriculum Links:
Biology & Chemistry
Key Stage 3
Key Stage 4

Core Practicals / RPs/ PAGs
AQA – RP7 Chy
Edexcel – 9.6C
OCR – PAG C5

Safety Precautions:

- Wear protective gloves and clothes, safety glasses.
- Assume that all red solutions are blood and handle according to safety regulations.
- After using the materials use the recipient for biological waste to discard them.
- Hazards of reagents: Table 3

Table 3 - Hazard of Reagents

Reagent	Hazard
Phenolphthalein	Carcinogenic Mutagenic Reproductive toxicity 
Sodium Hydroxide	Corrosive 
Metallic Zinc	Flammable Toxic to the environment 
Ethanol	Flammable 

Background Information:

The presence or absence of blood stains often provides important information for those investigating criminal cases. For this reason, forensic scientists are often called determine whether or not a particular stain is blood. Forensic scientists use chemical assays, such as presumptive blood tests, to reveal the blood presence. Subsequently further tests are carried out to confirm that it is effectively blood and whether it is of human or animal origin.

The chemistry employed in the presumptive tests is an oxidation-reduction reaction catalysed by the heme molecule, a factor of haemoglobin responsible not only for the transport functions (of oxygen and CO_2) as also by the characteristic red colour of the blood. The heme group is inserted in the category of "prosthetic groups", that is, non-peptide essential to certain proteins and includes not only an iron atom but also an organic part, the Tetrapyrrole Ring. The heme group catalyses oxidation-reduction reactions of various colourless substrates resulting in a colour change or chemiluminescence.

An oxidation-reduction reaction involves changes of the oxidation state. Specifically, the oxidation of a molecule means the molecule has lost electrons, and the reduction of a molecule means the molecule has gained electrons. In the presumptive tests, hydrogen peroxide is usually used as an oxidant, that is, an agent that causes oxidation (loss of electrons) of a given substance – reducing agent, staying with some of its electrons. The heme group functions as a catalyst for the oxidation-reduction reaction. A catalyst is a substance that increases the speed of a chemical reaction but is not affected or altered by the reaction.

What happens?

In the Luminol test, the luminol mixture is sprayed onto the suspected area and when the luminol comes into contact with the bloodstain there is an emission of bright blue light. The chemical reaction is an example of chemiluminescence. On the other hand, in the Kastle-Meyer test, when the colourless Kastle-Meyer solution is added to the red stain, it will turn a deep pink colour if blood is present.

How does it work?

Kastle-Meyer:

The Kastle-Meyer reaction consists of a reduced form of phenolphthalein and hydrogen peroxide, which react with each other to produce a pink solution made of water and a phenolphthalein ion [Figure 17].

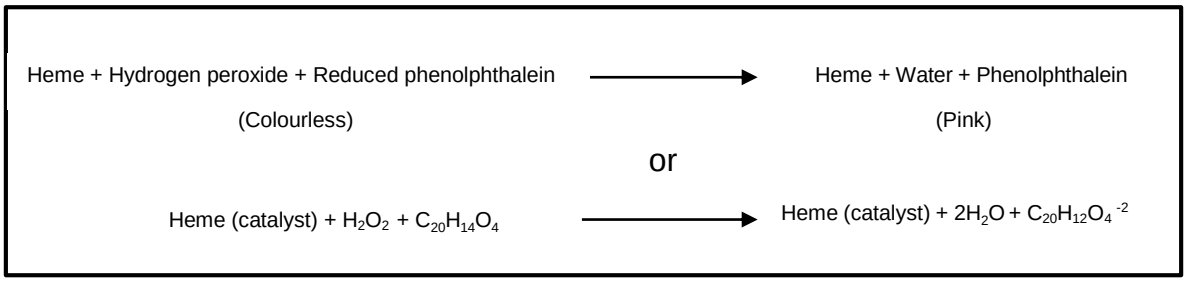


Figure 17 – Kastle -Meyer Reaction

The phenolphthalein has been modified from its conventional form by being reduced and pre-dissolved in alkaline solution, giving it a faint yellow colour. Then, in the presence of hydrogen peroxide (H_2O_2) in alkaline solution, the haemoglobin in the blood catalyses the oxidation of this form of phenolphthalein to its normal form ($C_{20}H_{12}O_4^{2-}$), which generates an intense pink colour.

Method for Kastle-Meyer Test

Materials in the Toolbox

- Kastle-Meyer Solution
- Hydrogen peroxide 3%
- Ethanol 96%
- Cotton Swabs
- Evidence#1
- Evidence#2
- Evidence#5
- Gloves
- Negative Control
- Positive Control

Materials you Provide

- Safety glasses
- Protective clothing
- Hotplate
- Digital scale
- 250 mL Graduated glass bottle
- 50 mL Boiling tube
- 250 mL Brown glass bottle
- 100 mL Graduated cylinder
- Watch glass
- Distilled water or deionized water
- Sodium Hydroxide
- Phenolphthalein powder
- Ethanol 96%
- Hydrogen peroxide 3%
- Zinc powder
- 2 known specimens (piece of cloth with animal blood and a simulated blood)

Notes for Technicians

Before starting the procedure, put a spot of animal blood (positive control) and simulated blood (negative control) on a section of clothing. Before testing any unknown stains, it is important to check all reagents on a known sample of blood. If you do not get the expected results with blood, then the reagents need replacing. Animal blood from liver obtained from the supermarket will suffice as the postitive control. You can make your own Simulated Blood using the following method.

Method for the preparation of the Simulated Blood:

a) Put on your gloves and protective clothing.
b) Place 2g of chocolate powder in a beaker and add 2 mL of hot water and stir vigorously until the chocolate dissolves.
c) Add to the mixture 8g of honey and 2 drops of red food coloring.
d) *Another alternative, would be to use tomato ketchup*

As an option the teacher can use the positive and negative control provided in the Toolbox.

Carrying out the Kastle-Meyer test:

1. Put on your gloves and protective clothing.
2. Wet a cotton swab with two drops of ethanol 96% and gently rub the wet swab on the known bloodstain (provided by the teacher).
3. Drop three drops of Kastle-Meyer solution onto the swab.
4. Drop three drops of hydrogen peroxide 3% onto the swab.
5. A positive pink colour will appear within seconds if blood is present.
6. Using clean cotton swabs, repeat steps 2 to 4 for the Evidence#5.
7. For Evidence#1, Evidence#2, Positive and Negative Control (provided in the Toolbox) put three drops of ethanol 96% then three of Kastle-Meyer solution and finally three drops of hydrogen peroxide onto the swab.
8. Record your results in Table 4.

After performing the test, discuss with students what results can be obtained using Table 4. In the 'Results' column, the students should describe whether they see a pink colour or not. In the 'Interpretation of the results' column, students should understand that if a pink colour appears, there is blood; and if there is no pink colour, blood is absent.

Table 4 - Table of test results.

Stains	Results	Interpretation of the results
Blood stain (positive control)	*Pink colour*	*Blood*
Simulated blood (negative control)	*No pink colour*	*No blood*
Evidence#1	*Pink colour*	*Blood*
Evidence#2	*No pink colour*	*No blood*
Evidence#5	*Pink colour*	*Blood*

Notes for Technicians

Method for the preparation of the Kastle-Meyer Solution (60 minutes)

1. Put on safety glasses, gloves and protective clothing.
2. Measure 90ml of distilled or deionized water with a graduated cylinder and transfer it to the graduated bottle.
3. In a watch glass, weigh out 20g of sodium hydroxide and add it to the beaker in small portions, swirling until the solid dissolves and the reaction is cold.

Caution: this process is very exothermic, so use a container with cool water and place the bottle inside to cool.

4. In a fume cupboard, weigh out 1g of phenolphthalein powder, add it to a boiling tube with 10ml of ethanol 100%, and swirl until the powder dissolves. (96% ethanol will suffice in the absence of 100%)
5. Add the prepared mixture to the sodium hydroxide solution. The solution turns bright pink.
6. Weigh out 20g of zinc powder and add to the beaker.
7. Place the beaker on a hotplate at the maximum temperature until it reaches a boil (approximately 15 minutes).

Caution: this process should be made in a fume cupboard, due to the vapours which are released.

8. Reduce the heat (approximately 160°C) and allow the solution to simmer until the bright pink solution turns colourless (or a very pale straw yellow), which may require approximately 30 minutes.
9. After the solution turns colourless, remove the beaker from the heat and allow it to cool to room temperature.
10. Carefully transfer the solution into a brown glass storage bottle labelled “Kastle-Meyer reagent” but take care not to transfer the zinc powder.

Caution: wet zinc powder is pyrophoric (catches fire spontaneously). See below note about disposal

CLEAPSS recommends the following to dispose of zinc powder:

- Rinse the zinc powder first, to make sure there's no Kastle-Meyer solution on it, as it's very alkaline so would use a lot more ethanoic acid
- Slowly add 1M ethanoic acid. Heat or spray may be produced. Test solution with indicator and add more ethanoic acid until the mixture is just acidic. Pour the neutralised mixture down the foul-water drain with further dilution.
- If the zinc powder catches fire, smother with clean, dry sand.

This solution remains usable for several months if stored at room temperature in a tightly stoppered bottle, and for a year if refrigerated.

Questions and Answers:

1. Explain why you need to use both a positive and negative control before testing the unknown stains:

Positive Control

Used to verify that all chemical reagents are functioning in the expected manner. If the expected colour change does not occur using the positive control, then new reagents are needed before testing any unknowns.

Negative Control

Used to verify that a pink colour will not result when using these reagents. If a pink colour is produced with the negative control, you need to get new reagents. There could possibly be some contamination of your reagents.

2. Explain how it is possible to get a positive reaction with the Kastle-Meyer using pig blood, if animal blood is different from human blood.

The Kastle-Meyer test detects the presence of heme molecule found in the haemoglobin of the blood. Both pigs and humans have haemoglobin in their blood. Therefore, both pigs and human blood will produce a positive reaction using the Kastle-Meyer and Luminol tests.

3. Explain why in the Kastle-Meyer test the reagents aren't directly applied to the original bloodstain.

It is important to preserve the evidence and avoid any contamination of the original piece of evidence.

4. In the Kastle-Meyer test, the pink colour should first be evident:

a) when applying the Kastle-Meyer solution to the cotton swab.
b) when applying the hydrogen peroxide to the cotton swab.
Explain your answer.

The pink colour should only be visible after adding the hydrogen peroxide. If the pink colour appears after adding the phenolphthalein, something is wrong. A new test should be performed. The pink colour occurs after the oxygen is released from the hydrogen peroxide. It is the heme molecule that acts as the catalyst causing the hydrogen peroxide to break down.

Activity II: Blood Typing Analysis

Objectives:

- Examine the ABO arrangement of blood using simulated blood and antiserum
- Determine the blood type of the evidences
- Describe the reaction (antigen – antibody) that occurs when typing blood
- Explain the red blood cells agglutination process
- Explain how this these methods help forensic investigators

Curriculum Links:

Biology & Chemistry

Key Stage 3

Key Stage 4

Core Practicals / RPs/ PAGs

AQA – RP7 Chy
Edexcel – 9.6C
OCR – PAG B2 & C5

Time required: 20 minutes

Safety Precautions:

- Wear protective gloves and clothes and safety glasses
- Assume that all red solutions are blood and handle according to safety regulations.
- After using the materials use the recipient for biological waste to discard them.
- Hazards of reagents in Table 5.

Table 5 – Hazard of Reagents.

Reagent	Hazard
Barium Nitrate	Harmful Irritant Skin Sensitizer 
Silver Nitrate	Oxidising Agent Corrosive Toxic to the Environment 
Sodium Metasilicate Pentahydrate	Toxic Corrosive 

Background Information:

Blood is usually the main trace found at a crime scene and is composed of a liquid portion called plasma, which contains mostly water along with dissolved nutrients, minerals, and oxygen. Suspended in the plasma are solid material consisting mainly of several types of cells: red blood cells, white blood cells and platelets.

The blood is considered a class evidence because many different people share the same blood type. By typing the blood found at a crime scene, it is possible to link a suspect to a crime scene or to exclude a suspect. However, matching blood types does not prove guilt since many people share the same blood type.

The blood type of a particular person depends on the presence or absence of certain antigens (usually a protein) found embedded within the cell or in the plasma membranes of red blood cells.

How does it work?

The presence or absence of A and B antigens on red blood cells determines a person's ABO blood type. This leads to the identification of four main blood types: A, B, AB (when both antigens are present) and O (when neither antigen is present). A third important blood antigen is the Rh factor. People with the Rh factor are Rh positive, and those who lack it are Rh negative [Figure 18].

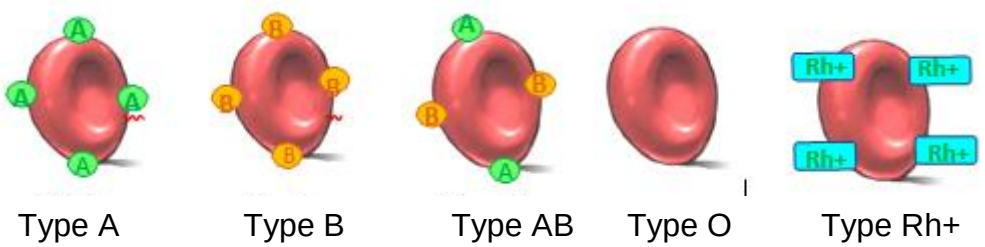


Figure 18 – Diagram showing the different human ABO blood types and Rh factor.

Blood types are determined by using antibodies (such as anti-A, Anti-B and Anti-Rh) that respectively react with the A, B and Rh antigens.

Antibodies, also known as immunoglobulins, are large Y-shaped protein molecules produced by plasma cells and used in the immune system. The antibodies bind to the molecular shape of an antigen, fitting like two complementary puzzle pieces [Figure 19].

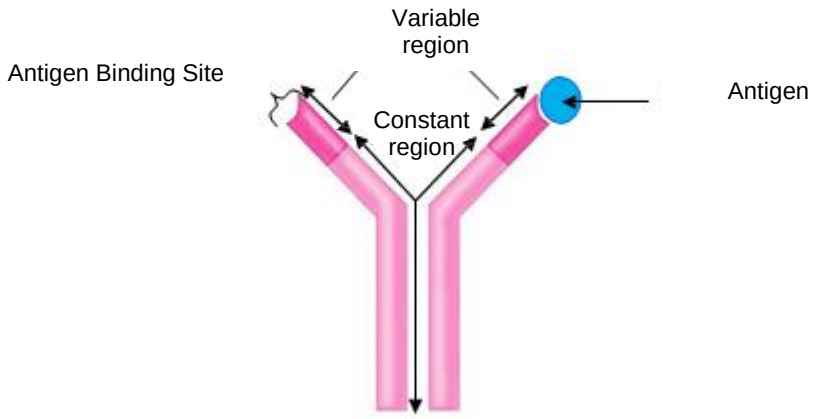


Figure 19 - The general structure of an antibody with an antigen in the binding site.

When antibodies and antigens of the same type (e.g. anti-A and antigen A) come together, one arm of the Y-shaped antibody attaches to the red blood cell and the second arm of the Y attaches to another red blood cell, and agglutination, or clumping, of the red blood cells takes place [Figure 20].

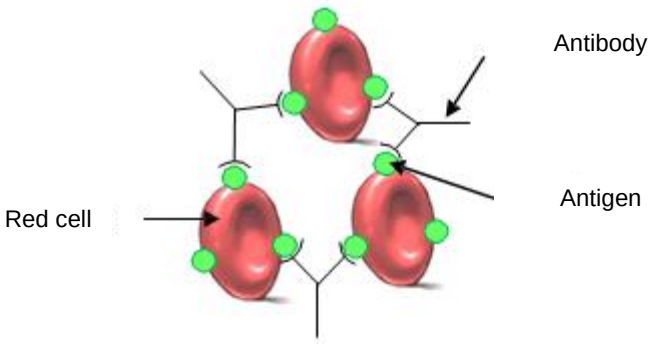


Figure 20 - An agglutination reaction between antibodies and the antigens on cell surfaces.

Method for determining blood type

Materials in the Toolbox

- Simulated Blood of Victim
- Simulated Blood of Suspect 1
- Simulated Blood of Suspect 2
- Simulated Blood of Suspect 3
- Simulated Blood of Evidence#4
- Simulated Blood of Evidence#5
- Simulated Anti-A Serum
- Simulated Anti-B Serum
- Simulated Anti-Rh Serum
- Blood Trays
- Toothpicks
- Gloves

Materials you provide

- Protective clothing
- Permanent marker
- Digital Scale
- 50 mL Boiling Tubes
- Watch glass
- Spatula
- 100mL beaker
- 100mL graduated cylinder
- Drop bottles
- Sodium Chloride (NaCl)
- Barium Nitrate ($Ba(NO_3)_2$)
- Silver Nitrate ($AgNO_3$)
- *Sodium Metasilicate Pentahydrate ($Na_2SiO_3.5H_2O$)
- Red food colouring
- Yellow food colouring
- Blue food colouring
- Green food colouring
- Distilled water or deionized water

Supplier of Sodium Metasilicate Pentahydrate ($Na_2SiO_3.5H_2O$): SLS Industrial stock it (states UK customers only) but is not listed in their school catalogue. https://www.scientificlabs.co.uk/product/71746-250G

Notes for Teachers

Before performing the test, shake the simulated blood and antisera in order to homogenise the solutions.

Notes for Technicians

1. Put on your gloves and protective clothing.
2. Use a permanent marker pen to label the blood trays as shown in [Figure 21].

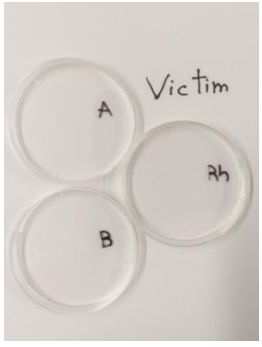


Figure 21 - Wells for testing

3. Repeat the process for the remaining samples:

    a) Tray 1: Evidence#4
    b) Tray 2: Evidence#5
    c) Tray 3: Victim
    d) Tray 4: Suspect 1
    e) Tray 5: Suspect 2
    f) Tray 6: Suspect 3

4. To determine the type of blood found in the Evidence#4, place 4 drops of simulated blood of Evidence#4 in each of the A, B and Rh wells of tray 1.
5. Repeat the process for Suspects 1, 2 and 3, the Victim and Evidence#5.
6. Add 4 drops of Anti-A serum (blue bottle) to each of the six wells labelled A.
7. Add 4 drops of Anti-B serum (yellow bottle) to each of the six wells labelled B.
8. Add 4 drops of Anti-Rh (green bottle) to each of the six wells labelled Rh.
9. Obtain three toothpicks for each slide. Stir each sample of anti-serum and blood with a separate clean toothpick for 30 seconds. To avoid spattering the simulated blood, do not press too hard on the typing tray.
10. Observe each tray and record your results in Table 6. Based on the agglutination, determine and record each blood type [Figure 22].

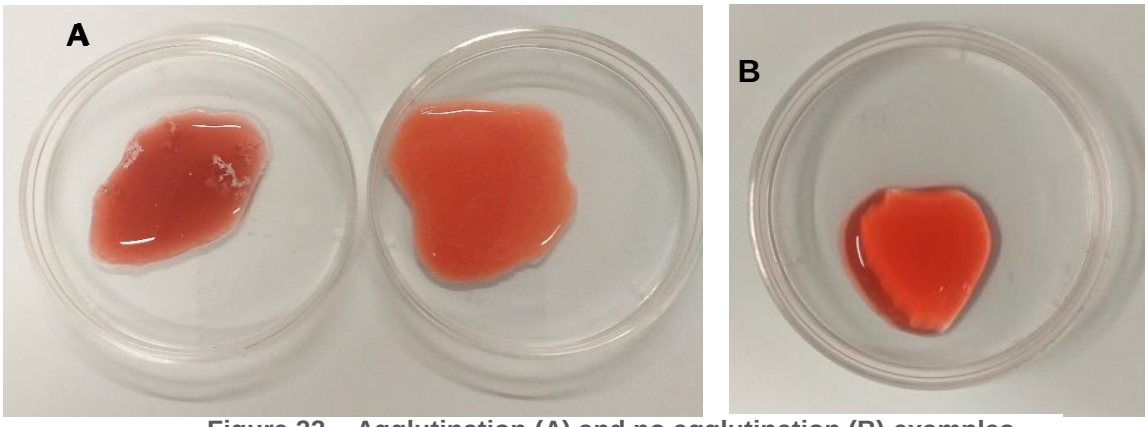


Figure 22 – Agglutination (A) and no agglutination (B) examples

Notes for Teachers

After performing the test, discuss with students what results can be obtained using Table 6. In the 'Results' column, students should describe whether they see agglutination or not. In the 'Interpretation of the results' column, students should indicate the type of blood based on the agglutination results. **What information does this yield for solving Case 1?**

Table 6 - Table of Test Results – with expected results

Stains	Agglutination? Yes/No	Interpretation of the results
Evidence#4	***Well A: no*** ***Well B: no*** ***Well Rh: no***	***O–***
Evidence#5	***Well A: yes*** ***Well B: no*** ***Well Rh: yes***	***A+***
Victim	***Well A: yes*** ***Well B: no*** ***Well Rh: yes***	***A+***
Suspect 1	***Well A: no*** ***Well B: no*** ***Well Rh: no***	***O–***
Suspect 2	***Well A: yes*** ***Well B: yes*** ***Well Rh: ye***	***AB+***
Suspect 3	***Well A: no*** ***Well B: no*** ***Well Rh: no***	***O–***

You can make your own simulated blood and antisera using the following method, which takes approximately 30 minutes.

Using these simulated blood and antisera (provided in the Toolbox), you can create a new case study with other blood groups.

Method for the preparation of simulated blood and antisera

1. Put on your safety glasses, gloves and protective clothing.
2. For the preparation of the simulated blood:

    a) Label four boiling tubes as “Type A”, “Type B”, “Type AB” and “Type O”.
    b) Weigh out 0.5 mol/L Sodium Chloride (NaCl) (0.73g/25 mL water) and add to the tube “Type A”.
    c) Weigh out 0.1 mol/L Barium Nitrate (Ba $(NO_3)_2$) (0.68g/ 25mL water) and add to the tube “Type B”.
    d) Weigh out 0.5 mol/L Sodium Chloride (NaCl) (0.73g) and 0.1 mol/L Barium Nitrate ($Ba(NO_3)_2$) (0.68g) with 25 mL of water and add to the tube “Type AB”.
    e) Add 25 mL of water to the tube “Type O”.
    f) To each tube add 8 drops of red food colouring and stir gently.

3. For the preparation of the simulated antisera:

    a) Label three boiling tubes as “Anti A”, “Anti B” and “Anti Rh”.
    b) Weigh out 0.1 mol/L Silver Nitrate ($AgNO_3$) (0.43 g/25mL water) and add to the tube “Anti A”.
    c) Weigh out 5% Sodium Metasilicate Pentahydrate ($Na_2SiO_3.5H_2O$) (2.17g/25mL water) and add to the tube “Anti B” and stir.
    d) Weigh out 0.1 mol/L Silver Nitrate ($AgNO_3$) (0.43 g/25mL water) and add to the tube “Anti Rh”.

e) To the tube “Anti A” add 1 drop of blue food colouring, to the tube “Anti B” add 4 drop of yellow food colouring and to the tube “Anti Rh” add 2 drop of green food colouring whilst gently stirring the tubes.

4. Transfer the simulated blood and antisera to identified drop bottles.

Questions and Answers:

1. Based on your results, does the Evidence#4 blood match the blood type of any of the three suspects or victim?

   ☒ **Yes** ☐ **No**

   Explain your answer.

   The blood type of Evidence#4 is O – and the suspects 1 and 3 have the same blood type.

2. Based on your results, does the Evidence#5 blood match the blood type of any of the three suspects or victim?

   ☒ **Yes** ☐ **No**

   Explain your answer.

   The blood type of Evidence#5 is A+ and the victim have the same blood type.

3. Explain the following sentence: "When blood from one of the suspects matches the crime-scene blood that does not prove that the suspect is guilty".

   This is not conclusive evidence because other people can have a similar blood type. If eyewitness accounts place the suspect at the crime scene, it does reinforce the case against him/her. If sufficient blood were available for a DNA analysis, this would reinforce his/her presence at the crime scene.

4. Blood types are determined by the presence of antigens located on:

   a) **All of the blood cells**
   b) **White blood cells**
   c) **T-helper cells**
   d) **Red blood cells**

5. If a person has type O+ blood, then they have:

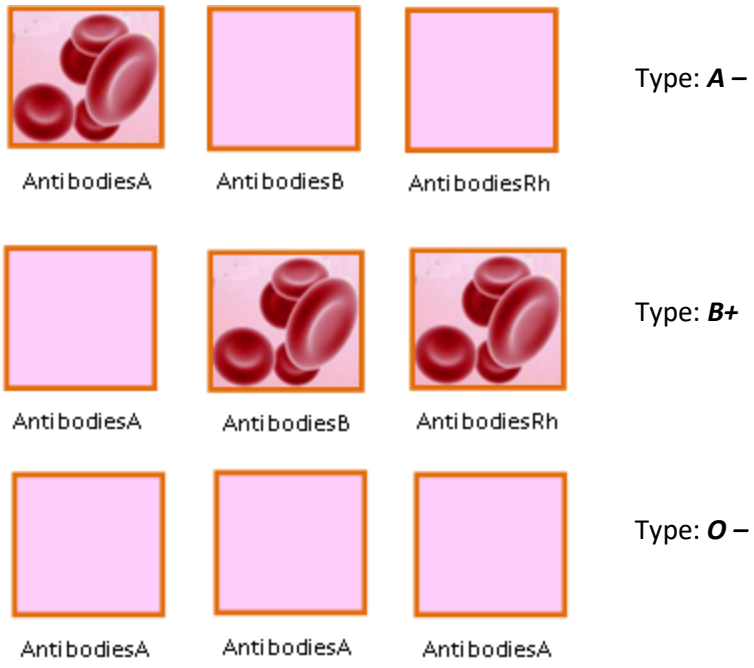
   e) O A and B antigens, but lack the Rh antigen

   f) An O antigen but not the Rh antigen

   g) None of the ABO nor Rh antigen

   h) The Rh antigen but not the A or the B antigens

6. For the following question, determine the blood type being tested. Indicate if the person is type A, B, AB or O. Be sure to indicate if the person is Rh+ or Rh- for each blood test shown below.



7. Explain why it is necessary to type the victim's blood when trying to determine if any of the blood found at the crime scene belongs to a particular suspect.

The victim's blood needs to be excluded from the crime scene evidence. If the victim's blood and the crime scene blood are the same type, further testing would be needed.

Activity III: DNA Profiling

Objective:

Using electrophoresis to compare DNA fingerprints to determine if they match.

Curriculum Links:

Biology: Key Stage 3

Physics: Key Stage 3

Physics: Key Stage 4

Time required: 90 minutes

Core Practicals / RPs/ PAGs

AQA – RP12
Edexcel – 2.11
OCR – PAG C3

Safety Precautions:

- None of the chemicals used in this activity present any special hazard, but it is still good practice to wear gloves and protective clothes while working with any chemical. The battery stack used in the gel electrophoresis exposes potential dangerous voltage and presents a fire hazard if you allow the positive and negative leads to contact each other.

Background Information:

Deoxyribonucleic Acid (DNA) is a large polymeric molecule found in the nuclei of practically every cell in the body, with the exception of red blood cells and nerve cells.

Every DNA molecule is made up of two strands of nucleotides (also called bases) that are twisted around each other to form a double helix. Each strand is made up of one nucleotide that joins to the other strand nucleotide in the middle forming a base pair. These base pairs are constituted by one of the following sets: Adenine (A)-Thymine (T) or Guanine (G)-Cytosine (C) [Figure 23].

Figure 23 - Base pair sequence

Each strand of DNA is made up of chains of nucleotides, half of which were donated by the mother and the other half by the father. Due to the way cells divide, DNA is unique to each individual, very much like fingerprints. The likelihood that any two people will inherit the same combination of nucleotides is very slim. For this reason, DNA fingerprints can be used to identify individuals.

DNA fingerprinting may be performed on extracted DNA from relatively small samples of cells, such as blood drops, saliva, hair follicles, skin or semen. It is therefore possible to distinguish between individuals by analysing patterns in their DNA. When performed under properly controlled conditions and accurately interpreted, DNA fingerprinting can link or exclude a suspect to a particular incident.

To create a DNA fingerprint, scientists first cut the long molecule into shorter segments using a polymerase chain reaction (PCR). The DNA segments are separated by size on an agarose gel in the process of gel electrophoresis. Gel electrophoresis is conceptually similar to chromatography, but with a slightly different goal. Normally, chromatography is used to separate different compounds from a mixture. With DNA gel electrophoresis, the goal is to separate DNA fragments according to different sizes.

What happens?

In order to visualize the different DNA fragments, an electrophoresis gel is made, and these samples are loaded into the agarose gel and placed in a salt solution. On this gel, an electric current is applied, causing the migration of the DNA through the gel [Figure 24]. The electrophoresis gel separates the DNA fragments by size, since shorter fragments move faster than larger fragments through the cross-linked structure of the gel.

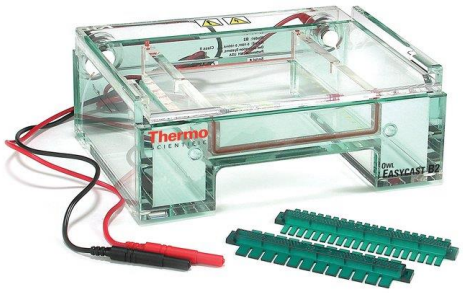


Figure 24 – Typical Gel electrophoresis apparatus

How does it work?

In the electrophoresis gel, the DNA samples (generated by PCR) to be analysed are loaded into wells formed in the gel (moulded from a jelly-like material, such as agarose). The gel is then immersed in a buffer solution, which maintains the pH and carries the electric current, and is subjected to an electric current. Since the DNA fragments have a negative overall charge due to the phosphate groups, the DNA samples are attracted to the positive electrode (the anode), which is positioned at the end of the gel, furthest from the wells containing the DNA solution.

The gel selectively delays the migration of the DNA fragments toward the positive electrode. Small DNA fragments pass through the gel relatively unhindered, and so travel a greater distance through the gel. Larger fragments move proportionally more slowly because the gel provides more resistance to their progress. The positions of the various fragments provide a graphical map of the fragment size distribution in the specimen [Figure 25].

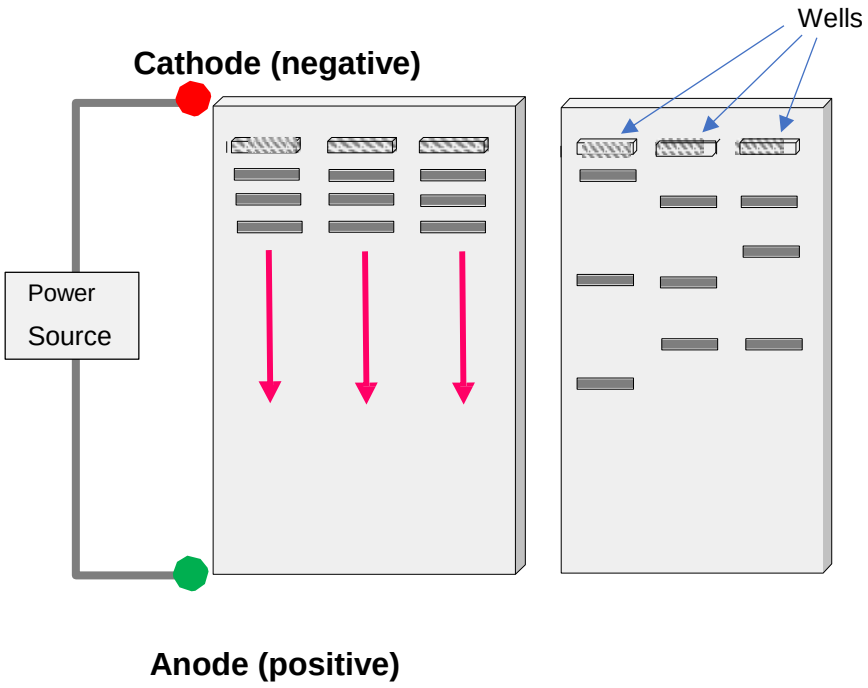


Figure 25 - DNA fragments separated by size

Method for carrying out gel electrophoresis

Materials in the Toolbox

- DNA samples (Evidence#4, Suspect 1, Suspect 3, Victim)
- Corn Starch
- Sodium Bicarbonate
- Alligator clips
- Paper squares
- Plastic container (12cmx18cm)
- Comb
- Gloves

Materials you provide

- Protective clothing
- Spatula
- Scissors
- Scalpel
- Adhesive Tape
- 250 mL beaker
- 100 mL graduated cylinder
- Watch Glass
- 9V Batteries
- Digital Scale
- Microwave oven
- Distilled water
- Tweezers
- 4 x Carbon rods

Notes for Technicians

The comb used in this procedure is supplied in the Toolbox, but you can make your own comb/s using the lid of a margarine tub. A template is provided below. *This may need adjusting, depending on the size of the tub being used to form the gel.*

The paper squares used in this procedure are supplied in the Toolbox, but you can make your own paper squares by cutting small squares (0.3 cm x 0.3 cm) from blank paper.

You can make your own DNA samples using gel food colouring. For Evidence#4, use green food colouring; for Suspect 1 use green food colouring; for suspect 3 use red food colouring and for victim use purple food colouring.

Part 1: Preparation of the gel

Notes for Technicians

It is suggested that a class set is prepared.

1. Put on your gloves and protective clothing.
2. Prepare the Sodium Bicarbonate buffer weighing, in a watch glass, 3g of Sodium Bicarbonate and measuring in a graduated cylinder 300ml of distilled water.
3. Use a plastic container as a tray for the gel (provided in the Toolbox) [Figure 26].

Figure 26 – Plastic container in which to form the gel

4. Transfer 180 ml of sodium bicarbonate buffer, previously prepared, to the graduated cylinder.
5. Weigh 23.4g of corn starch in the glass beaker and mix with 90ml of the sodium bicarbonate buffer and add the other 90ml after the corn starch is dissolved.
6. Put the mixture in the microwave for 30 seconds and then mix with a spatula. Put it back in the microwave and stop for mixing every time the mixture rises. Repeat this process for 60 seconds until the mixture is thick enough and does not fall off the spatula.

Caution: for this step use oven gloves to hold the glass beaker, once it gets too hot.

7. Put the heated mixture inside the plastic container and smooth the surface with a square of plastic cut from the lid of a margarine tub (or similar). Scrape the surface (wipe off excess) until the gel is as level and smooth as possible.
8. Place the comb 3cm from one end and make sure that the mixture is between the teeth of the comb [Figure 27]. *The long, middle teeth of the comb should not reach all the way to the bottom of the gel layer.*

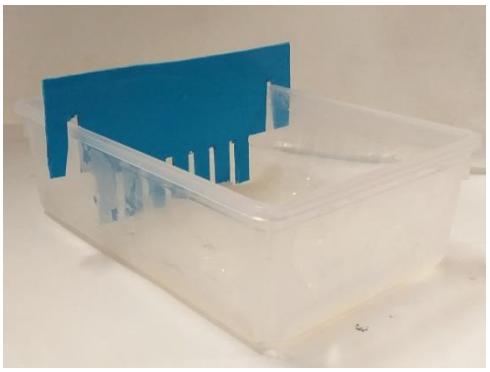


Figure 27 – Plastic container with Comb

9. Place the gel in the freezer for 20 minutes at -20ºC.
10. After 20 minutes, check the bottom of the container to see if it is still warm. If so, keep it in the freezer for 5 more minutes.
11. After taking out the gel from the freezer, carefully remove the comb.
12. With a scalpel, trim the gel so that it no longer touches the edges of the box – remove 1cm of gel from the sides, trim the top end to within 1 cm of the wells formed by the comb, and trim the bottom end so that it finishes within 3cm of the far end. **DIAGRAM**

Part 2: Load and Run the DNA Specimens

1. Trim two carbon rods so that the lie neatly at both short ends of the tub containing the gel rectangle, ensuring that they do not touch the gel.

2. Arrange two short carbon rods using an electrode holder (or similar) so that when balanced at either end of the tub, they make good contact with the horizontal carbon rods in the previous step.

Caution: The paper clips cannot touch the bottom of the gel because it appears a brown colour (due to cooking the flour).

Figure 29 –

3. With the tweezers, dip the end of the paper squares [Figure 30] in DNA samples (provided in the Toolbox), remove any excess with a paper towel and place them carefully inside the gel wells, taking care not to have any of the DNA touch the top surface of the gel.

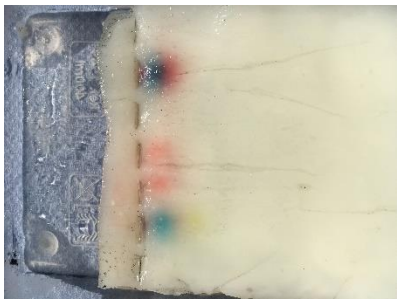
Caution: Clean the tweezers between samples with alcohol to prevent contamination.

Figure 30 – Paper Squares

4. Slowly and carefully fill the plastic container with sodium bicarbonate buffer until the top surface of the gel is just, but entirely, immersed in the buffer, with the level of the buffer a few millimetres above the surface of the gel. Do not pour buffer directly onto the gel surface, or you may raise the DNA samples out of the wells, rather use a pipette towards the end to carefully raise the level.
5. Connect five or seven 9-Volt batteries in series.
6. Connect power to the electrophoresis apparatus, making sure that the negative end of the batteries is connected to the carbon rods closest to the wells containing the DNA samples. [Figure 31].

Figure 31 - Turn on the power

7. You should immediately see bubbles rising from the carbon rods. If this is not the case, check all connections. Over time, you should be able to see the dyes migrating from the wells (negative terminal side) toward the far end of the gel (positive terminal side). Continue observing the progress for at least 45 minutes or until it's possible to distinguish between the strips. *There may be some debris accumulating within the solution from impurities in the carbon rods. If this is the case: at the end of the allocated time - disconnect the circuit, carefully pour the buffer solution away and gently rinse the gel rectangle in order to better observe the results. See picture below:*



8. Record your results in Table 7.

Notes for Teachers

After performing the test, discuss with students what results can be obtained using Table 7. In the 'Results' column, students should indicate how many bands they observe. In the 'Interpretation of the results' column, students should indicate the colour of each band.

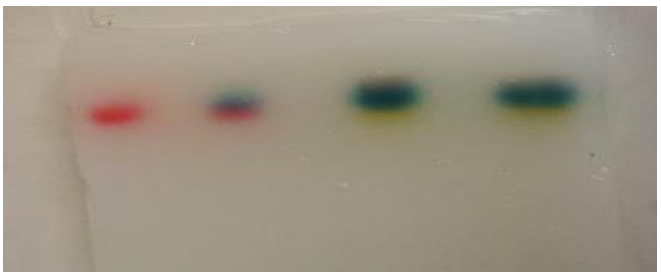
Schools that have all the equipment necessary to carry this out using an agarose electrophoresis gel can use the samples provided in the Toolbox.

Table 7 – Table of results

Samples	Results	Interpretation of the results
Evidence#4	*2 bands*	*Blue and Yellow*
Victim	*2 bands*	*Blue and Red*
Suspect 1	*2 bands*	*Blue and Yellow*
Suspect 3	*1 band*	*Red*

Notes for Teachers

Example of the electrophoresis gel using food colouring:



Suspect 3 Victim Evidence#4 Suspect 1

Questions and Answers:

1. Based on your results, did any of the studied samples have the same DNA profiling as Evidence#4?

   [X] **Yes** [ ] **No**

   Explain your answer.

   Evidence#4 and Suspect 1 have the same number of bands with the same colour. That means that both samples refer to the same DNA sample.

2. Gel electrophoresis:

   a) **Cannot separate DNA fragments**
   b) **Is similar to gas chromatography**
   c) **Has a very thin column for the stationary phase**
   d) **Uses an electric current as the mobile phase**

3. Explain the purpose of gel electrophoresis.

   The purpose is to separate DNA fragments of different sizes.

4. Explain the function of the power supply during gel electrophoresis.

The power supply allows the passage of electric current through DNA samples, causing the migration of the DNA through the gel.

Activity IV: Comparison of different fingerprint patterns

Objective:
Identify your fingerprints and compare them with your classmates

Time required:
20 minutes

Curriculum Links:
Biology: Key Stage 4

Core Practicals / RPs/ PAGs
OCR – PAG B1

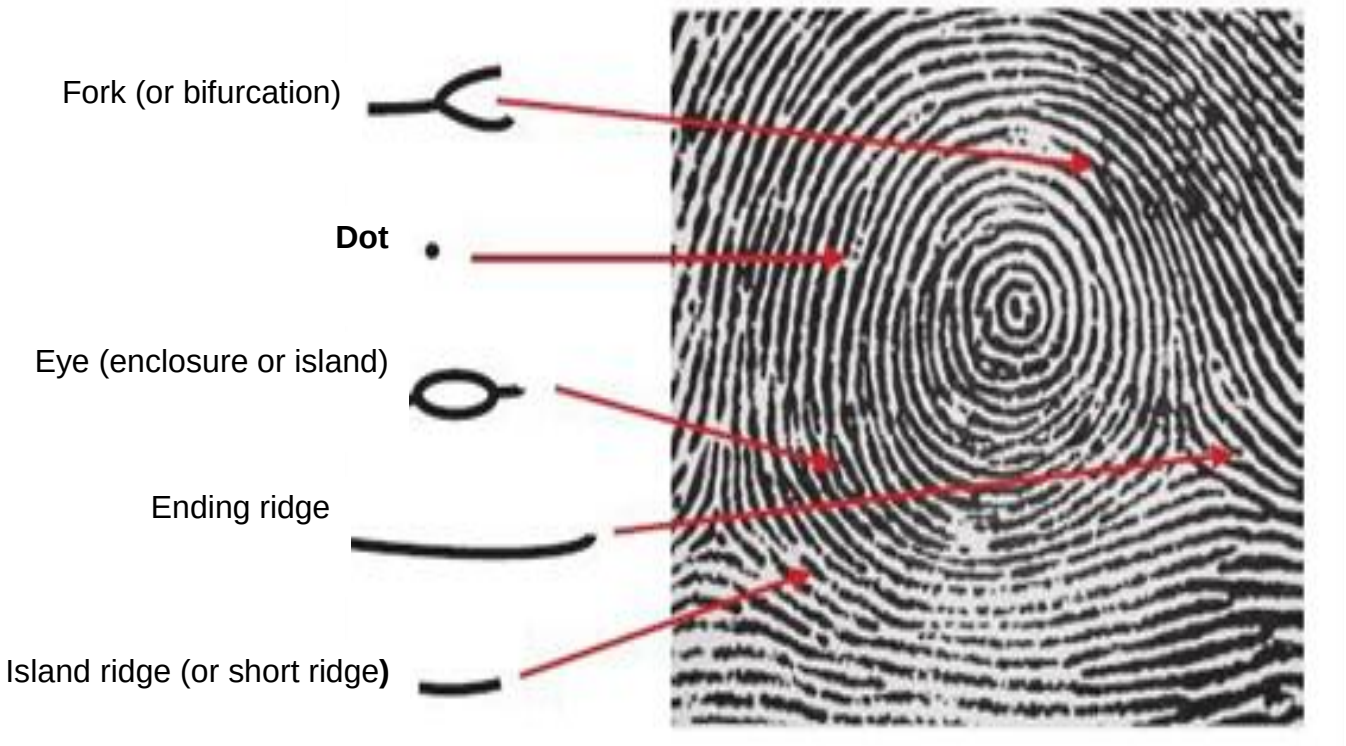
Background Information:

Many patterns and characteristics have been used to individually distinguish and identify humans. Fingerprints - marks present on the tips of human fingers - can be used for identification.

Fingerprints are static and do not change with age, so an individual will have the same fingerprint from infancy to adulthood. The friction ridge pattern of an individual forms between the 10th and 24th week of foetal development. The exact arrangement of the ridges is determined by the dermal papillae, a layer of cells that separates the outer layer of skin (the epidermis) from the underlying dermis. As the body grows, the pattern changes size but not shape. Since each person has a unique set of fingerprints, even identical twins, they can be used for individual identification.

The major ridges in each finger form a pattern. There are three major pattern types: arch, whorl and loop. These patterns are categorized based on the presence or absence of deltas [Figure 32].

The uniqueness of a fingerprint is not determined by its general shape or pattern but by a careful study of its ridge characteristics (also known as minutiae) [Figure 33]. The identity, number, and relative location of characteristics imparts



uniqueness to a fingerprint. If two fingerprints are to match, they must reveal characteristics that not only are identical, but also have the same relative location to one another in the print.

Figure 33 - Some minutiae patterns used to analyse fingerprints

What happens?

Ink is the most common method for recording friction ridges skin. Inked fingerprints are recorded on collection cards. Each fingerprint is rolled in ink and subsequently rolled into the corresponding box on the collection card. The finger is rolled from nail to nail to capture the entire friction ridge surface. This means the finger must be rolled from one side of the finger (at the nail) all the way around the other side of the nail. A fingerprint rolled from nail to nail should be roughly rectangular in shape.

Method for analysing fingerprint patterns

Materials in the Toolbox

- Magnifying glass
- Clear Adhesive Tape
- Ink pad
- Identification card
- Collection card

Materials you provide

- Pencil
- White paper

Part 1: Using pencil to create a graphite pad

1. On a blank piece of white paper, rub the graphite pencil in a back-and-forth motion, creating a patch of graphite of area about 5 by 7 centimetres.
2. Rub your right index finger across the graphite patch, gently rolling from side to side so that the fingertip becomes covered with graphite from the first joint in the finger to the tip, and from fingernail edge to fingernail edge.
3. With the tape provided in the Toolbox, carefully press the sticky side of the tape onto your finger from the edge of your fingernail across your finger to the other side of your fingernail.
4. Gently remove the tape.

5. Press the tape, sticky side down, into the collection card provided in the Toolbox.
6. Examine your fingerprint using a magnifying glass.
7. Compare your fingerprint to the pictured samples.
8. Identify your fingerprint pattern type (loop, arch, or whorl) and some minutiae patterns (fork, dot, eye, ending ridge, or island ridge).

Part 2: Using an ink pad

1. Rub your right index finger across the ink pad, rolling from side to side while applying pressure so that the fingertip becomes covered with ink from the first joint in the finger to the tip, and from fingernail edge to fingernail edge.
2. On the identification card, provided in the Toolbox, gently press your finger in the box indicated as right index finger from one edge of your fingernail to the other side of your fingernail.
3. Repeat the steps 1 and 2 for the other fingers, for both hands.
4. Examine your fingerprints using a magnifying glass.
5. Compare your fingerprints to the pictured samples.
6. Identify your fingerprint pattern type (loop, arch, or whorl) [Figure 34] and some minutiae patterns (fork, dot, eye, ending ridge, or island ridge).

Figure 34 - Types of fingerprint patterns

Part 3: Data collection from class

1. Count the number of students showing each of the three types of fingerprint patterns (for the thumb finger) and place those numbers in the Table 8.
2. Complete the rest of the Data in Table 8 with the requested information.

Table 8 – Data Collection from class

	Loop	Whorl	Arches
Number of students showing trait			
Total size of class (This will be the same total for each column)			
Percentage of class showing the trait (Divide the number of students with trait by the total size of class, then multiply by 100%)			
Experts say this percentage should be	**65%**	**30%**	**5%**

Questions

1. Did the class percentage agree with the value given by experts?

   ☐ **Yes** ☐ **No**

   Explain your answer using data for support.

2. Explain why fingerprints are an effective means of identification.

   Fingerprints are static and do not change with age, so an individual will have the same fingerprint from infancy to adulthood.

3. Fingerprints are formed:

   a) shortly after birth
   b) at about two years of age
   c) between 10 and 24 week's gestation
   d) at 30 weeks' pregnancy

4. Do identical twins have identical fingerprints?

☐ **Yes** ☒ **No**

5. The three main types of fingerprints are classified as:

a) loops, whorls, and deltas
b) whorls, bifurcations, and arches
c) loops, whorls, and arches
d) arches, core, and deltas

6. Explain why inked fingerprints are rolled from nail to nail.

Fingerprints are rolled from nail to nail in order for the entire fingerprint to be visible. This inked surface gives all the needed ridge characteristics for correct classification.

7. Classify each of the following prints as loop, whorl, or arch.


Type: ***Whorl***


Type: ***Arch***


Type: ***Loop***

Thematic: Human Individual Identification

Activity V: Dusting and Lifting Latent Fingerprints

Objective:

Understand how to recover and identify latent fingerprints with graphite powder

Curriculum Links:

Biology: Key Stage 4

Time required:

40 minutes

Core Practicals / RPs/ PAGs

N/A

Safety Precautions:

- Cover the work area with bench paper or newspaper.
- Handle the dusting powder with care, because it can be very messy.

Background Information:

Fingerprints are one of the most important types of clues found at a crime scene. The impressions left by fingerprints can be of three types: Patent (visible fingerprints deposited via a substrate such as blood or paint), Plastic (fingerprints left in a mouldable material), and Latent (hidden fingerprints formed by sweat and oils of the skin).

Latent fingerprints are the most frequently pursued type of fingerprint by forensic detectives and need to be visualized using appropriate development techniques (depending on the type of surface) before comparison and possible identification.

The oldest fingerprint development method is ‘dusting’. Fingerprint dusting is a method of enhancing latent fingerprints by applying powders to the fingerprint residues, making them visible. The powder granules adhere to the lines of sweat and oil left by the fingerprint ridge. Dusting a crime scene or item of evidence with fingerprint powder is a physical processing method rather than a chemical reaction.

Fingerprint powders are used primarily for dusting nonporous surfaces such as glass and polished metal, most commonly to reveal latent fingerprints on immovable objects at crime scenes. Although black powder is the most frequently used material, powders of other colours can be used to enhance a fingerprint's contrast against backgrounds of different shades.

What happens?

The development process of latent fingerprints should start with a non-destructive visual examination of the item of interest using oblique lighting (a beam of white light at oblique angles to the surface). Oblique lighting allows the observation of details on a surface where light and shadows are created by the light beam.

After the visual examination of the fingerprints, these will be revealed with a duster. Dusting involves the use of a soft brush to lightly coat a surface carrying a fingerprint with a powder (made from finely ground carbon, charcoal, titanium, or aluminium) and can provide excellent results if done skilfully. If not, dusting can easily damage or destroy any latent fingerprints present, so the brush is moved across the surface of the object without touching it. This allows the powder to adhere to the surface of the fingerprint residues without getting into the cracks in the surface [Figure 35- A].

The exposed fingerprint can then be lifted with adhesive material, such as fingerprint lifting tape, and placed on a paper card or a sheet of acetate as a permanent record [Figure 35– B].

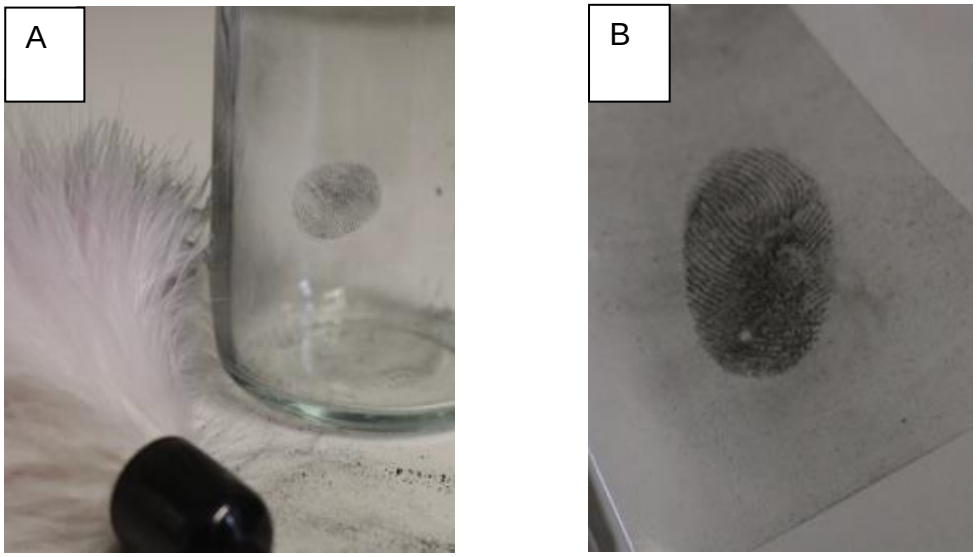


Figure 35 - Fingerprint revealed with dust (A) and collected with fingerprint lifting tape (B).

Method for lifting fingerprints

Materials in the Toolbox

- Magnifying glass
- Clear Adhesive Tape
- Fingerprint brush
- Graphite powder
- Collection card
- Gloves

Materials you provide

- Protective clothing
- Bench paper or newspaper
- Paper towel
- Evidence#3
- Evidence#4

Notes for Technicians

Evidence#3 and Evidence#4 are not provided in the Toolbox since it would be impractical to include a hammer and a knife in the box. These pieces of evidence can be replaced by other objects, such as a glass, clock glass or a piece of window glass.

To place a fingerprint on Evidence#3 and Evidence#4, follow these steps:

a) Wipe the object with a clean cloth or paper.
b) Take your thumb and run it along the side of your forehead, nose or the back of your neck. These areas of your body are rich in oils and will help lubricate the ridges of the thumb to produce a clear print.
c) Choose an area on the object and touch the object with your thumb. Use a paper towel or other type of cloth in your hand to prevent leaving other fingerprints. Be careful to avoid placing any other fingerprints in this area.

1. Cover the worktable with bench paper or newspaper.
2. Put on your gloves and protective clothing.
3. Put the graphite powder over the Evidence#4 and with the dusting brush placed between your hands, gently twist so that the feathers spin off the excess powder near the surface of the object you are dusting. A latent (hidden) fingerprint should begin to appear. Continue to dust lightly, touching the surface until you have exposed as much of the latent print as possible.

4. With the tape provided in the Toolbox, place it over the fingerprint and press down.
5. Peel off the tape and place it on the collection card, provided in the Toolbox. This process is called lifting the print.
6. Examine the fingerprint using a magnifying glass.
7. Identify the fingerprint pattern type (loop, arch, or whorl).
8. Repeat the process with Evidence#3 if you effectively provided it.

You can make your own graphite powder using 3 pencils. Cut the pencils longitudinally and take out the graphite. Grind the graphite until it becomes a fine powder.

Questions and Answers:

1. Indicate which type of surface is better for dusting.

   Fingerprint powders are used for dusting nonporous surfaces such as glass and polished metal.

2. The colour of powder used to dust fingerprints will vary depending of the colour of the surface.

   ☒ **True** ☐ **False**

3. Explain what oblique lighting is.

   Oblique light is a beam of white light at oblique angles to the surface.

4. Plastic fingerprints must be dusted or treated in order to identify the ridge patterns.

   ☐ **True** ☒ **False**

5. Fingerprints that are deposited via a substrate such as blood or paint are referred to as:

   a) plastic fingerprints
   b) patent fingerprints
   c) latent fingerprints
   d) indented fingerprints

Activity VI: Revealing Latent Fingerprints using Iodine Fuming

Objective:
To recover and identify latent fingerprints with chemical reagents

Curriculum Links:
Biology: Key Stage 4

Time required:
10 minutes

Core Practicals / RPs/ PAGs
N/A

Safety Precautions:

- Wear protective gloves and clothes
- Iodine crystals are toxic and corrosive and stain skin and clothing.
- Iodine vapours are toxic and irritating – carry this out within a fume cupboard
- ***To dispose of the papers carrying an iodine fingerprint, place in a solution of 1M sodium thiosulfate until they are colourless. Dispose of in normal waste. Any left-over iodine crystals can be disposed of in the same way, or recycled for a later activity.***

Background Information:

At a crime scene, investigators look for any clues that can help them identify the criminal. One of the most important types of clues is fingerprints. Some fingerprints are immediately visible, but others are not. The invisible fingerprints are described as latent.

A latent fingerprint is a fingerprint left on a surface as a result of the oils and sweat from the pores of the finger. The primary component of latent fingerprints is ordinary sweat. Sweat is mostly water and will dry after a fairly short period of time. The other components of latent fingerprints are primarily solid and can remain on a surface for a much longer period of time. These other components

include organic compounds like amino acids, glucose, lactic acid, peptides, ammonia, riboflavin, and isoagglutinogens, as well as inorganic chemicals like potassium, sodium, carbon trioxide, and chlorine.

These fingerprints need to be visualized using appropriate development techniques before comparison and possible identification. There are many chemical methods for making latent fingerprints visible, one of which is iodine fuming. This method is based on a chemical reaction between the fumes and the molecules that make up the fingerprints.

The iodine method is a non-destructive technique that uses iodine vapours for visualizing latent fingerprints on porous and nonporous surfaces, such as paper, index cards, magazines, and cardboard.

What happens?

In the iodine method, the object carrying latent fingerprints is normally suspended in a closed chamber in which warmed iodine crystals sublime directly into vapour, filling the air within the chamber. The result is brownish fingerprints.

How does it work?

Iodine:

Iodine is solid at room temperature. When heated it sublimes (it changes directly from the solid state to a gaseous one). When iodine fumes are exposed to fingerprint residues, specifically to lipids or fats in the residue, they react to form a brownish image of the fingerprint [Figure 36]. This image is only temporary because the iodine will continually sublime and evaporate as a gas into the air, leaving the fingerprint invisible, or latent, once again.

Figure 36 - Latent fingerprint revealed with iodine fuming

Method for revealing fingerprints using Iodine fuming

Materials in the Toolbox

- Magnifying glass
- Iodine Crystals
- Evidence#6
- Gloves

Materials you provide

- Protective clothing
- Scissors
- Adhesive tape
- Tweezers
- Narrow strip filter papers
- Stoppered boiling tubes

Notes for Technicians

Prepare the boiling tubes in advance of the session by adding 3 or 4 iodine crystals to each tube. Stopper each boiling tube firmly and store in the fume cupboard.

To repeat this activity, you can make your own paper card and follow these steps:

- Take your thumb and run it along the side of your forehead, nose or the back of your neck. These areas of your body are rich in oils and will help lubricate the ridges of the thumb to produce a clear print.
- Choose an area on the paper card and touch the paper with your thumb. Be careful to avoid placing any other fingerprints in this area.

Procedure

1. Put on gloves and protective clothing.
2. Collect a stoppered boiling tube containing iodine crystals from the fume cupboard.
3. Working within the fume cupboard, transfer Evidence#6 to the boiling tube, using tweezers and immediately replace the stopper. (Evidence#6 = paper found in the athlete's sports bag)
4. The crystals sublime, filling the boiling tube with iodine vapour. Latent prints should start becoming visible within a few seconds to a few minutes, as faint orange smudges on the specimen. [Figure 37].

Figure 37 – Latent fingerprint revealed with iodine fuming

5. Leaving the paper within the boiling tube, examine the fingerprint using a magnifying glass.

Questions and Answers:

1. Explain what a latent fingerprint is.

A latent fingerprint is a fingerprint left on a surface as a result of the oils and sweat from the pores of the finger.

2. Explain for which types of surfaces iodine fuming are appropriate.

The iodine fuming is appropriated for porous and nonporous surfaces, such as paper, index cards, magazines, and cardboard.

3. Describe and explainthe appearance of the fingerprint when it reacts with iodine fuming:

Brownish fingerprint.

This is because iodine is a brown solid which sublimes to a gas, then reforms to a solid as it comes into contact with the latent fingerprint.

CHAPTER 2:
ENVIRONMENT EDUCATION

Palynology

Objectives

With this chapter you will be able to:

- Recognize general characteristics of pollen grains
- Realise the variety of plants that exist in correlation with the pollen grains variety
- Explain that the different seasons in which plants flower influences the type of pollen grain found in a crime scene
- Explain the role of ecology of a plant as a possible indication of a location for a crime scene

Botany is a very broad area of study, which includes the analysis of wood, fruit, seed, branches, leaves, plant hairs, **pollen**, spores, and algae cells.

Palynology is the science that studies the palynomorphs, that is, **pollen**, plant spores, fungal spores and other microscopic entities resistant to acid degradation.

Forensic Palynology refers to the study of palynomorphs to obtain probatory evidence in criminal cases. Palynomorphs adhere very easily to various types of surfaces, without being noticed, often referred to as "silent evidence". They are very resistant to degradation and difficult to remove off surfaces, even after successive washes.

Pollen is a granular powder produced by plants with seeds (Angiosperms and Gymnosperms) with the purpose of sexual reproduction during the flowering season associated with the species. The male gametophyte on the pollen grain is wrapped and protected by a hard coating that protects it in its journey from the stamen (male organ) of a flower to the carpel (female organ) of another flower. The transfer of pollen can occur through the aid of animals (mainly bees) or through environmental factors (wind and water) allowing pollination to occur.

Morphology of Pollen Grains

The pollen grain has a wall with two distinct layers: the exine and the intine. The exine is made of sporopolenin (a polymer very resistant to degradation that protects against mechanical actions and dehydration) and a small amount of polysaccharides, while the intine is made of cellulose similar to the wall of other plant cells.

Figure 38 shows a cross section through the wall of a pollen grain.

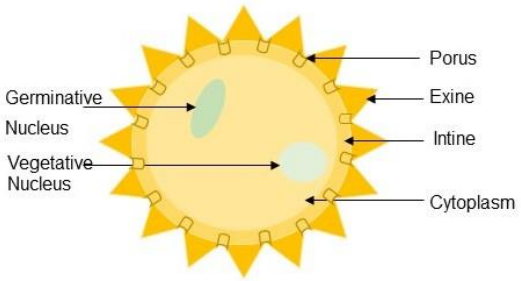


Figure 38 - Constitution of the wall of a pollen.

Due to the resistant nature of sporopollenin, the exine layer is able to withstand the passage of time, even in adverse conditions.

Other morphological characteristics are important such as size; if they are isolated or grouped; the shape; the number, shape, position and structure of pollen grain openings are also important in identifying the plant species to which they belong [Figure 39].

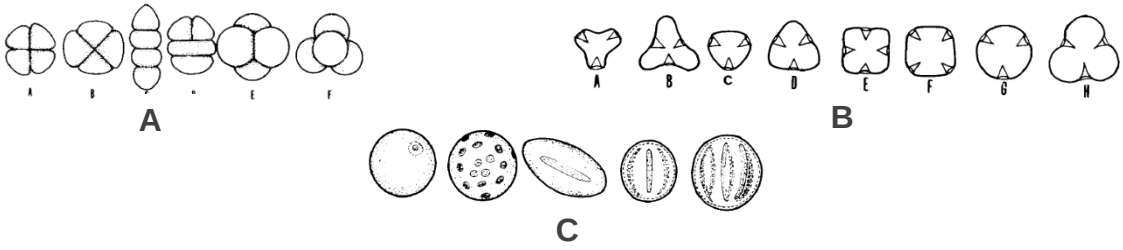


Figure 39 - Representative images: of the types of arrangement of the pollen grains in the tetrad (A); of the triangular, quadrangular and circular forms of the pollen grains (B); and the shape and number of openings of the pollen grains (C)

Analysing the type of pollen found on a victim, their clothing, or other objects can tell forensic investigators where they have been – this can be applied to illicit drugs, money and even food. It can be possible to trace items to a specific location, or even estimate the time of year at which a crime took place.

Entomology

Objectives

With this chapter you will be able to:

- Explain the importance of insect's life cycle and other arthropods in the estimation of Post-Mortem Interval (PMI)
- Explain that environmental conditions also affect the PMI

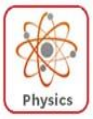
Forensic Entomology is the study of insects and other arthropods (through taxonomy, biology and ecology) that inhabit decaying remains to aid criminal investigations.

The earliest recorded application of this method has been found in China, in the 12th century. From the 19th century, through the publication of the book of Jean Pierre Mégnin, this science began to give more emphasis to criminal investigation. This book describes the various successions of insects in the cadaver and is considered one of the most important publications in Forensic Entomology.

The Importance of the Life Cycle of Insects in PMI

The rate of insect development is directly dependent on environmental conditions (temperature, relative humidity, wind speed, precipitation), especially temperature. The higher the temperature, the faster the insects will develop. If the ambient temperature during the development period is known, the time since the death of the body, or Post-Mortem Interval (PMI) can be determined. However, there are several factors that affect the duration of insect development and which complicate the estimation of PMI. These include the heat generated by the cluster of larvae in the body; the tissue from which they feed (which can vary the rate of development of the larvae); the presence of toxic substances; obstructions that prevent the access of adult flies to the body (for example, buried bodies, submerged, wrapped in plastics) and unfavourable environmental conditions. These and other factors have to be considered when estimating the PMI.

Hair and Footprints



Objectives

With this chapter you will be able to:

- State the importance of hair evidence in criminal investigations
- State the importance of footprints in criminal investigations

Hair

Hair is a protein filament that grows from follicles found in the dermis and is one of the defining characteristics of mammals. The length, colour, shape, root appearance, and internal microscopic characteristics distinguish one species of mammal from another.

Hair identification is an important tool, used not only by forensic researchers, but also by wildlife biologists, archaeologists, and anthropologists.

The hairs can be transferred during physical contact, so their presence may associate a suspect with a victim or a suspect at a crime scene. The types of hair recovered, the condition and the number of hairs found all have an impact on their value as evidence in a criminal investigation. In addition, hairs may still be important in a forensic context since pollen and spores may be retained indefinitely, depending on the frequency of washing.

Hair can be analysed to test the existence of drugs or nutritional deficiencies in a person's system, to determine its chemical composition or to extract DNA from the hair follicle, They can also be analysed by comparing the microscopic characteristics of the hairs, helping to determine the species of the animal whose hair was recovered.

Footprints

The footprints are an indication of presence, useful for knowing the number of animals or people that have passed in a certain place. They are also important

evidence found at the crime scene, as they allow reconstruction of crime events (determining the direction of travel or the number of suspects) and may also be important in determining if the body has been moved from the crime scene, or to identify whether any animals have eaten the dead body.

It is not only the size and pattern of a footprint which matters. The material that adheres to footprint, like soil, provides a variety of forensic information (hair analysis, pollen grains, fibre analysis etc.). All of this can help determine what may have happened, when and where.

Footprint characterization

The observation of animals is very difficult, - they may be very wary of humans, or are only active at night. However, the footprints of these animals can provide information about the species, numbers and habits of animals that live or pass through the area under investigation. In order to characterize the footprints, attention must be paid to the digits (ie the fingers of the animals), the number of digits (how many), the shape (if they are round or elongated, for example), the presence or absence of nails and to the size and shape of the cushions/pads (although they are not present in animal with hulls). This information facilitates the identification of species [Figure 40]. At the forensic level it is important to compare the pattern present in the footprint and its size, since it can guide the identification of suspects present at the crime scene. Another more specific use may be in identifying vehicle tyre tracks [Figure 41].

Figure 40 – Examples of animal footprint

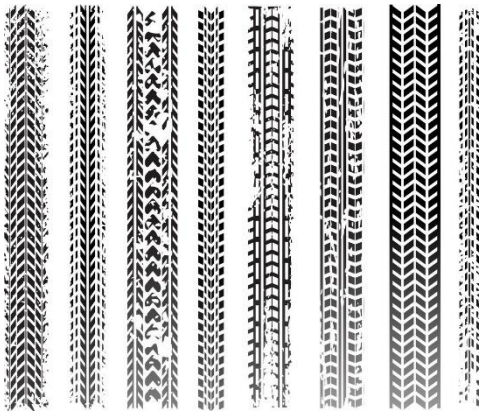


Figure 41 – Examples of tyre tracks

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Real Life Case Studies

Mellory Manning murder (2008)

Figure 42 – Mellory Manning.

Manning was strangled, beaten and stabbed to death before being dumped in Christchurch's Avon River, where she was found on 19 December 2008. Police had cut squares of cloth from different areas of Manning's cardigan to allow Dallas Mildenhall (World renowned pollen scientist) to map the pollen distribution, in the hope it would tell them something about how she was killed.

The samples confirmed that Manning had died on her back. But they also showed something else. Something startling.

One of the pollen species Mildenhall found in Manning's cardigan came from ripgut brome grass. Like most pollen types, it has a characteristic structure, which in this case includes a single pore. But in about 10 per cent of the grass grains Mildenhall saw pollen which had two pores, the second being smaller and not symmetrically arranged as in most multi-pore pollen structures. It was a genetic mutant. If he could find the same structures in any of the samples from possible crime scenes he would know where Manning had been killed.

With a new sense of urgency, Mildenhall continued to work through slides of pollen gathered by local police officers. Eventually, he struck lucky, finding a sample which showed the same mutant pollen grains, which had been gathered from a yard owned by a gang known as the ‘Mongrel Mob’, near to where Manning’s body had been found. Other samples from that weedy yard, confirmed this mutation.

The discovery was a breakthrough for the Manning case. Mob prospect Mauha Huatahi Fawcett was eventually found guilty of Manning's murder, although there is currently a retrial taking place for this crime.

Insect Informants

Figure 43 – Larvae and pupae of two different fly species.

On a mid-November afternoon, police were called to investigate a foul-smelling smell emanating from a single-family home in the south-eastern United States. It did not take long for the investigating officers to discover a shallow grave in the dirt basement of the house, which contained the badly decomposed body of a young female.

It was immediately apparent that the victim had died of a single bullet wound to the head inflicted by a small caliber rifle. A scrupulous examination of the corpse and excavation of the soil in and around the grave site by a forensic entomologist working with police revealed the presence of numerous larvae and pupae of two different fly species.

The specimens were collected from the scene and brought back to the laboratory to be reared. Supplemental information, including weather data and soil temperature, was also analysed in order to determine the principal climatic conditions at the death site. Based on the developmental biology of both species of flies in that environment, the forensic entomologist estimated that the specimens associated with the body the longest were in their fourth stage of development. It was estimated that the victim had died approximately 28 days prior to the date her body was discovered.

This specific information allowed the authorities to target their investigation in and around the estimated time of death. Within a short time, they identified a female suspect who eventually confessed to having killed the victim precisely 28 days prior to the time the body was found. She further admitted to attempting to bury the victim in a shallow grave in the basement of the house after committing the homicide.

Calculating the developmental rate of the flies provided investigators with the only scientifically reliable method of estimating the time of the victim's death and subsequently led to the arrest and conviction of the killer.

Cold Case Murder Solved by a Hair (1977)

Figure 44 – Frank Wright.

In August 2010, Riverside County sheriff's homicide detectives announced that they had solved the 1977 murder of a Rubidoux bartender, James Anagnos, who was known as "Jimmy the Greek,".

Jimmy Anagnos was found dead on October 18, 1977 inside his bar at closing time. He had been stabbed 20 times with a steak knife and had been hit four times in the head with a blunt object. Hair was found clenched in Anagnos' hand, and was believed to have come from the suspect, Frank Wright, who had been arguing with Anagnos about an hour before the bar closed.

At the time, the forensic laboratory was not to confirm that the hair was a match for Wright, and the case against him was not able to be made. There were no independent witnesses to the attack, so the District Attorney's Office did not file charges against Wright.

In 2010, the hair was sent to a private laboratory in Texas for DNA testing. The DNA test showed that the hair did indeed belong to Frank Wright, who had died in 2002 from complications related to alcohol abuse. Another example of forensic science helping to solve even historic cold cases.

ACTIVITIES

Activity VII: The Hidden Secrets of Pollen Grains

Objective:

Observe and distinguish the characteristics of pollen.

Realize the variety of plants that exist.

Explain the significance of the different seasons in which some plants flower

Curriculum Links:

Biology: Key Stage 4

Core Practicals / RPs/ PAGs

AQA – RP1 Biology
Edexcel – 1.6
OCR – PAG B1

Required time: 15 minutes

Safety Precautions

- Ensure those with allergies do not come into contact with pollen
- Use gloves in the preparation of pollen samples for observation

Background Information:

Just as fingerprints are specific to individual people, each type of pollen is specific to each species of plant. Analysing the pollen on clothing, footwear or objects can give forensic investigators useful information about both suspects and victims – including whether there are any connections between them, where they have been etc. Although analysis of pollen grains can help determine locations which may be of interest to forensic investigators, if the plants are very common, have a very large dispersion area, or a long flowering season, this can complicate matters. Therefore, not all pollens are good forensic indicators.

Method for analysing Pollen grains

Materials in the Toolbox

- Pasteur Pipette
- Evidence#14 (Pollen samples of *Pinus pinaster*)
- Gloves

Materials you provide

- Microscope
- Distilled water
- Slides
- Cover slips
- Brush

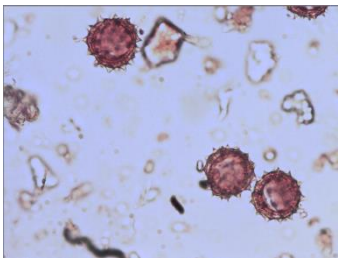
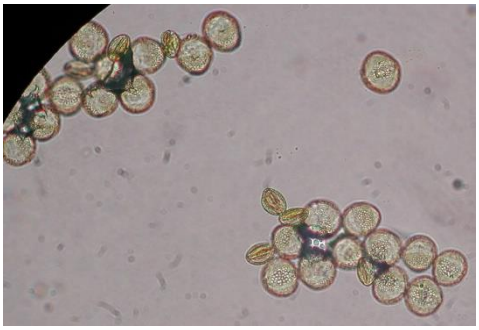
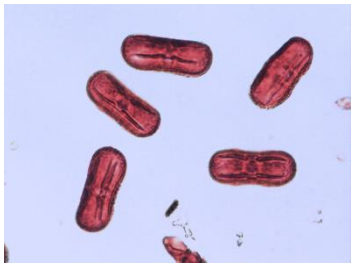
1. Put on your gloves and protective clothing.
2. Put a drop of distilled water with a Pasteur pipette on a slide.
3. With the brush take a small sample of pollen (Evidence#14) then tap the brush above the water on the slide to release the pollen.
4. Place the cover slip on top. Absorb any excess water that may be present at the edges of the cover slip using blotting paper.
5. Place the preparation under the microscope and observe. In Table 9 record which image of the pollen grain corresponds to the observed evidence.

Notes for Teachers

If you don't have access to the Toolbox material you can adapt Table 9 information, changing the images of the pollen grains to the species that you could provide. Use a pollen grain data base such as https://www.paldat.org/search/A to note the Ecology and Flowering characteristics for students.

Students could also collect and observe different types of pollen grains from local species.

Table 9 - Record of Observation Results

Image of the Pollen Grain	Evidence nº	Species
		*Anthemis cótula* Ecology Annual meadows, often in agricultural fields, crops, orchards and uncultivated. Flowering Between April and October
		*Salix atrocinerea* Ecology Margins of watercourses and ponds. In moist soils, sometimes nitrified. With somewhat acidophilic preferences. Flowering Between January and June
		*Angelica sylvestris* Ecology Wet meadows of tall grasses, usually in dark places, sometimes at the edge of water lines. Flowering Between May and October
		*Polygala myrtifolia* Ecology Shrub used for ornamental purposes, usually associated with garden areas. Flowering In temperate / warm weather practically all year round.
	X	*Pinus pinaster* Ecology In pine forests or mixed forest stands. In acid soils, mainly sandy near the coast, but also on shales in interior zones. Flowering Between January and July

Questions and Answers

1. Consider the information you have noted in Table 10. What conclusions can you draw regarding where the victim may have been before he died? Look back over the case history – does this corroborate other observations made that the original crime scene may not have been in the forest?

Before he died, it is likely that the victim had been in a location with a garden area and a freshwater pond. This is because the species Polygala myrtifolia is a shrub normally used for ornamental purposes in gardens, and the species Salix atrocinerea is associated with the margin of watercourses.

The case history noted "trailing marks" suggesting that the body may have been moved or dragged to forest and that this was not the original crime scene.

2. Considering the pollen grains present in Evidence#14, are you able to draw any conclusions as to when the crime took place? Explain your response.

The evidence associated with the crime suggests that it occurred somewhere between January and July, because coincide their flowering season.

Activity VIII: The Insect Clock

Objectives:

To explain the importance of the life cycle of a blowfly in determining the time of death of a corpse

Observe and distinguish different orders of insects.

To appreciate the influence of environmental factors on decomposition.

To graph data and derive conclusions

Curriculum Links:

Biology: Key Stage 4

Core Practicals / RPs/ PAGs

N/A

Time required: 45 minutes

Safety Precautions

- None – this is a paper-based activity

Background Information:

Forensic entomology consists of the use of insects (and other arthropods) to aid in the criminal investigation, since they are usually the first to find the cadaver. The main application of this science is to calculate the time since death occurred, known as the Post-Mortem Interval (PMI). This is achieved through the study of the insects which have invaded the body, how advanced they are in their lifecycle, how many generations of insects there have been, etc – all of which depend on the time which has elapsed since death.

The first insects to find the corpse are a member of the fly family. Their eggs hatch into larvae, which feed on the dead tissue. See Figure 45 for the life cycle of a fly.

Figure 45 - Life Cycle of a Fly

Phases of Decomposition

There are four main stages to the decomposition of a cadaver (corpse). There are:

- 1st phase: Fresh
- 2nd phase: Bloat
- 3rd phase: Active decay
- 4th phase: Advanced decay
- 5th phase: Dry (leading to skeletonization)

The succession of insects is related to the five phases of decomposition of the cadaver (1st phase: initial or fresh, 2nd phase: chromatic, 3rd phase: emphysematous, 4th phase: liquefaction and 5th phase: skeletonization) [Figure 46].

Figure 46 - Stages of poultry decomposition: (A) Initial Phase or Fresh Phase (B) Bloat phase (C) Active Decay phase, (D) Advanced Decay phase and (E) Skeletonization phase

Different insects may interact with the cadaver depending which decay phase it is in. The first insects to interact with the cadaver are Diptera flies as they can detect odours over long distances. The larval stage of this fly uses the dead body as a source of food. Coleoptera beetles are characteristic of later stages of decomposition, where the larval stage has greater capacity of adaptation and where the larvae of the Diptera flies can also serve as food.

The surrounding environmental conditions affect the decomposition rate of the body. Temperature, precipitation, wind speed and relative humidity are all factors which the forensic investigators need to take into account. The location where the body was found must be considered, along with the presence of clothing or chemicals. All of these may affect the speed at which decomposition takes place.

Activity to investigate the effect of temperature on maggot growth

Material in the Toolbox

- Evidence#12 (Life Cycle of the Fly)

Materials you provide

- Worksheets
- Graph paper

Watch this clip from Nature: https://www.youtube.com/watch?v=mj3-8Axkqe4

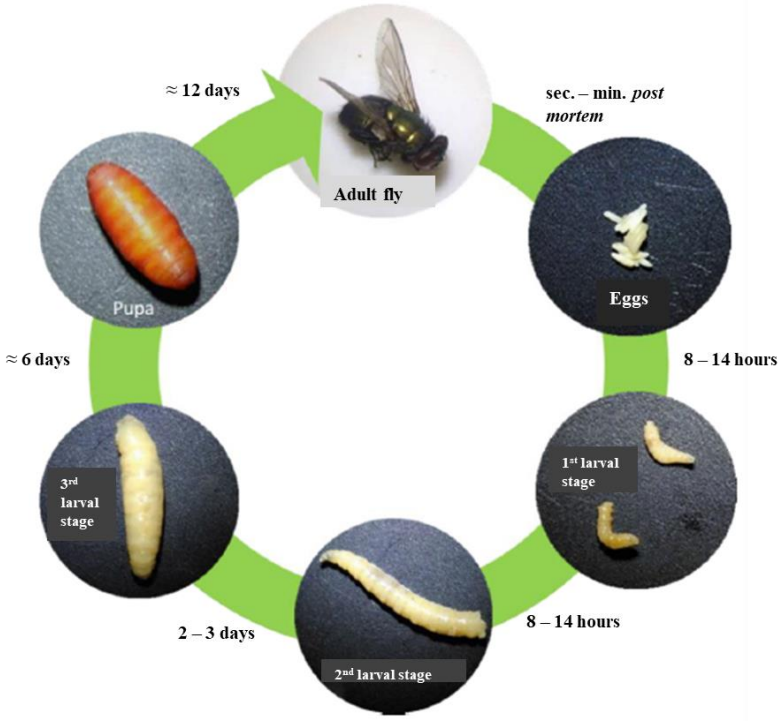
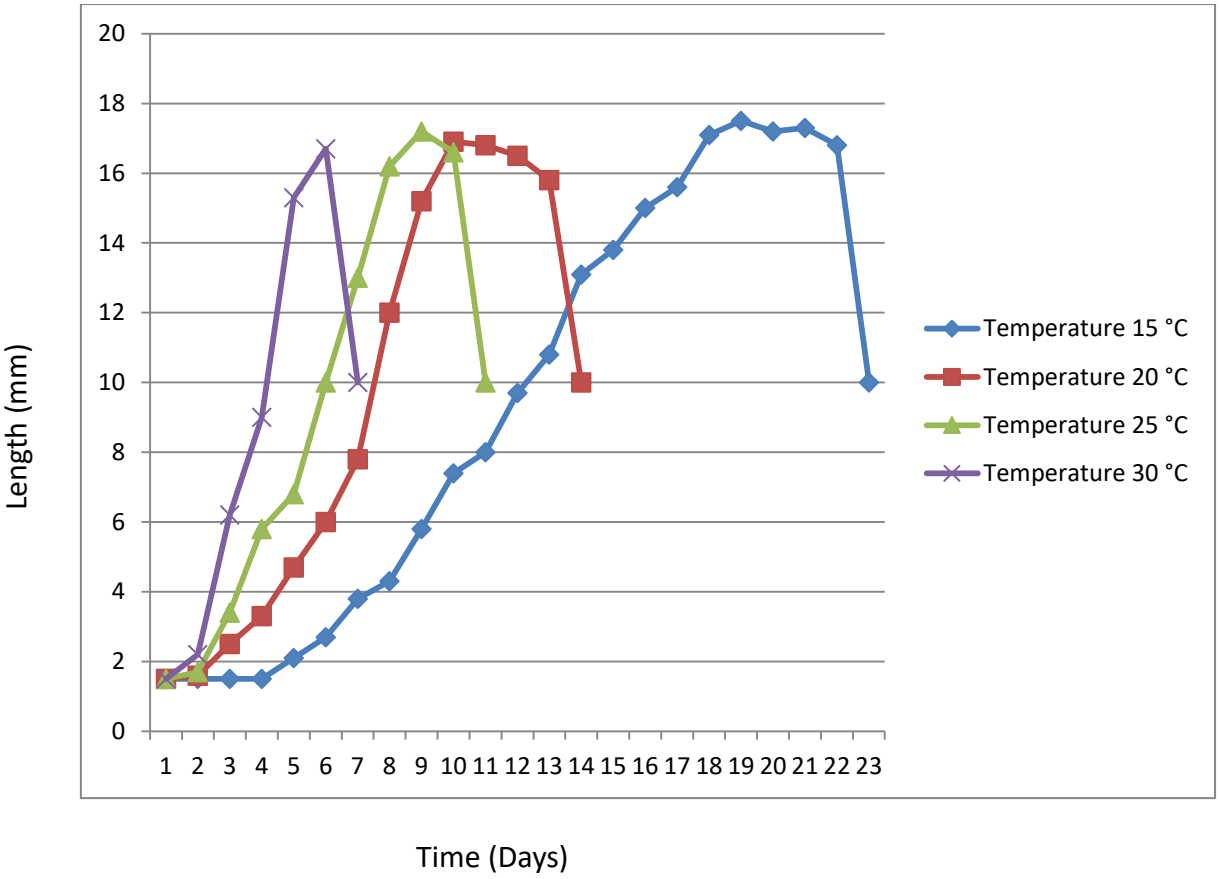


Figure 47 – Example of Life Cycle of a Fly

Crime Scene Investigators take the temperature at the crime scene, as the growth of maggots varies with temperature. Use the data supplied to make comparison graphs of larvae growth with time. Draw all curves on one set of axis. Then, answer the questions!

Time after egg-laying (in days)	Temperature 15 °C	Temperature 20 °C	Temperature 25 °C	Temperature 30 °C
0	1.5	1.5	1.5	1.5
1	1.5	1.6	1.7	2.2
2	1.5	2.5	3.4	6.2
3	1.5	3.3	5.8	9.0
4	2.1	4.7	6.8	15.3
5	2.7	6.0	10.0	16.7
6	3.8	7.8	13.0	10.0
7	4.3	12.0	16.2	
8	5.8	15.2	17.2	
9	7.4	16.9	16.6	
10	8.0	16.8	10.0	
11	9.7	16.5		
12	10.8	15.8		
13	13.1	10.0		
14	13.8			
15	15.0			
16	15.6			
17	17.1			
18	17.5			
19	17.2			
20	17.3			
21	16.8			
22	10.0			

Example of graph students should obtain



Questions and suggested answers on data:

1. Why did the forensic entomologist stop taking readings at day 22 for the larvae at 15 °C, day 13 for larvae at 20 °C, day 10 for larvae at 25 °C and day 6 for larvae at 30 °C?

   The larvae had turned into pupae and hence did not grow any more.

2. Describe the growth of the larvae at each of the different temperatures.

   The larvae initially hatched to the same size (1.5mm). They then grew in size at a steady rate until they reached approximately 17mm. They then slowly grew shorter but all suddenly lost length in one day until all reached a length of 10mm.

As they started to change to pupae they became shorter but fatter, so the mass went into becoming fatter in girth

3. Describe the effect of temperature on the growth of the larvae. Explain your answer

   As the temperature increased the time taken to reach maximum length decreased as the larvae grew quicker.

   The biggest difference appears to be between 15 and 20ºC (9 days difference) with smaller intervals in the remainder (3 days between 20 and 25ºC, 4 days between 25 and 30ºC)

Questions and Answers

1. What is the function of the Forensic Entomologist?

   Study of the life cycle of insects found on a cadaver for an estimate of the Post-Mortem Interval.

2. Describe the phases of decomposition of the cadaver.

   1st phase: initial or fresh, 2nd phase: chromatic, 3rd phase: emphysematous, 4th phase: liquefaction and 5th phase: skeletonization

3. What are the two major orders of insects used in Forensic Entomology investigations?

   Diptera and Coleoptera

4. Define PMI?

   Post-Mortem Interval.

5. Indicate three factors that may affect the PMI.

   [Suggestion]

   Climate, presence of clothing, type of environment where the cadaver is found.

Activity IX: Hair and Footprints

Objective:

Recall the difference between human and non-human hair.

Identify species from their footprint

Explain the importance of distribution and conservation status.

Curriculum Links

Key Stage 3:

Biology, Chemistry, Physics

Key Stage 4: Biology

Core Practicals / RPs/ PAGs

AQA – RP1 Biology
Edexcel – 1.6
OCR – PAG B1

Time required: 60 minutes

Safety Precautions

- Wear gloves and lab coat on footprint activity

Background Information:

A hair consists of three main anatomical regions: the cuticle, cortex and when present, medulla [Figure 51]. An entire hair should contain a portion of a root and an axis [Figure 52].

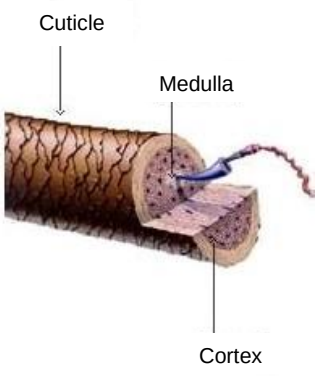


Figure 51 - Anatomical diagram of a hair

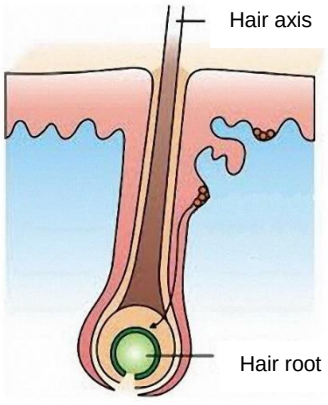


Figure 52 – Complete hair

In a forensic investigation, when some of the evidence is hair, the first question that must be answered is whether the hair is of human or non-human origin [Figure 53]. In the attempt to identify non-human hair, aspects related to the three anatomical regions, and other characteristics, should be studied in a systematic way.

Figure 53 - (A) Deer family hair; (B) Dog's hair; (C) Human's Hair

In Table 10 is a summary of the major differences between hairs of human and non-human origin.

Table 10 - Differences between Human and Non-Human Hairs

Feature	Human Hair	Non-Human Hair
Colour	Relatively consistent along the axis	Often shows tonal/colour variation along the axis
Cortex	Occupies most of the axis width-larger than the medulla	Usually less than the width of the medulla
Pigment distribution	Equivalent, though slightly larger in the direction of the cuticle	Central or denser in the direction of the medulla
Medulla	Less than a third of the axis width. Amorphous, mostly non-continuous when present	Greater than a third of the axis width. Continuous, often varying in the aspect along the axis and with defined structure
Scales	Overlap in a similar way along the axis, from the root to the tip	Mostly present variation of overlap along the root axis to the tip

A microscopic examination of hair at 4x, 10x and 40x allows you to record the general shape of the hair; whether the root is present and if so, its shape and appearance; the basic characteristics of the hair shaft, the type of medulla that it has [Figure 54] and even whether the hair has sustained some damage.

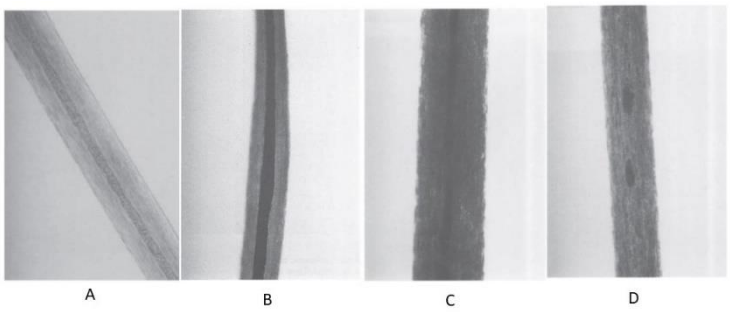


Figure 54 - Appearance of some types of medulla: (A) continuous, translucent; (B) continuous, opaque; (C) greater medulla area than the non-medullary cortex; (D) lower medulla area than the non-medullary cortex

Another type of forensic analysis that can be performed on hair is nuclear DNA analysis. This can only be done on hair that was plucked by force and during its growth phase (anagen) [Figure 55]. Only under these conditions will cells from the sheath or follicular tissue remain attached to the root zone for possible analysis [Figure 56]. Unfortunately, in most crime scenes, the hair found is devoid of this material, and it is not possible to analyze nuclear DNA.

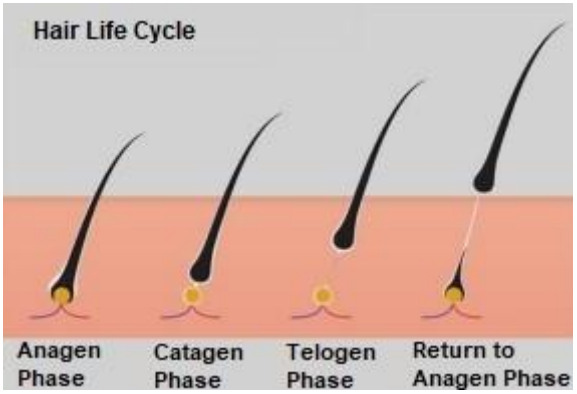


Figure 55 – Phases of Hair Life Cycle

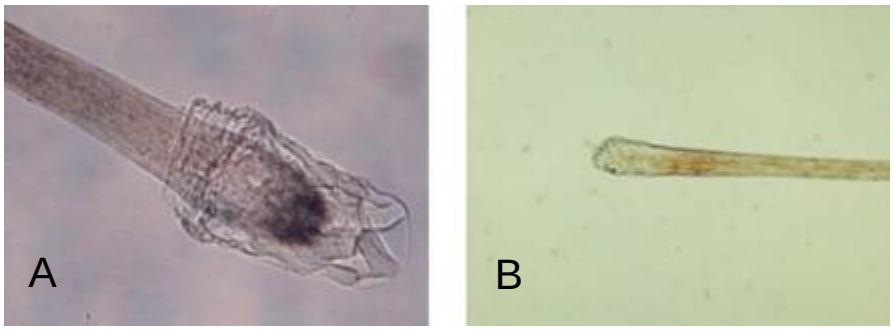


Figure 56 - (A) Hair root that allows the analysis of nuclear DNA; (B) Hair root that does not allow nuclear DNA analysis

Method for analysing hair and footprints

Material in the Toolbox: Hair

- Cat hair
- Dog hair
- Pasteur pipette

Material in the Toolbox: Footprints

- Footprints in resin (Evidence#11)
- Gloves

Material you provide: Hair

- Evidence#13
- Slides
- Cover slips
- Tweezers
- Water
- Microscope

Material you provide: Footprints

- Plastic container
- Tablespoons
- Water bottle with water
- Newspaper (3 sheets)
- 200ml measuring cylinder
- Toothbrush
- Plaster

Notes for Technicians

For Evidence#13 you need to provide either human or animal hair. Using Table 10 students will try to determine its origin.

Notes for Teachers

A complementary exercise for students is to compare their own hair to that of their peers, as well as to the animal hair provided. Are they sufficiently skilled to determine whether two hairs are from the same student?

Microscopic observation of hair using two complementary techniques: The cuticular impression and the transverse section of the medulla

The cuticular impression

1. On one slide, brush with varnish and then put a hair on the varnish.
2. Wait a few seconds, until the varnish dries, and remove the hair carefully with the help of tweezers.
3. Place the slide under the microscope and observe the mark left by the hair.
4. Next we check if the observed pattern coincides with some known pattern.

Easy Microscopic observation of hair

1. On a slide put a drop of water with the Pasteur pipette, and place the Evidence#13. (Try to place the root part in the drop first - if too long, cut only at the tip end).
2. Put the cover slip on top.
3. Place under microscope and observe at various magnifications.
4. Make notes of the characteristics you are observing in the hair.
5. Use Table 10 to try to identify if the hair is of human or non-human origin.

Footprints

Notes for Teachers

The next experiment consists of using wolf (Canis lupus signatus) footprints and comparing them.

Evidence#11 will correspond to the footprints that will be collected from the ground by the students with the plaster cast.

A complementary activity would be to make an impression in the ground with a shoe before the classroom activity, and have students collect a foot print from it, and try to discover to whom it belongs.

Notes for Technicians

Make the footprints casts:

Look for a place with soil preferably moist and without plants:

1. Put on gloves and protective clothing during the experiment.
2. Remove any plants and water dry soil until it looks muddy [Figure 57].

Figure 57 - Put water in a dry soil location (on the left), which looks muddy (on the right)

3. Place the resin footprint (Evidence#11) on the soil and press them slightly to make sure they are marked and then carefully remove them [Figure 58].

Figure 58 - Footprints in resin (left) and placed on the ground (right)

Preparing the plaster:

1. Mix 12 tablespoons of plaster with 150ml of water (measured with a graduated cylinder), to prepare the wolf footprint.
2. Put the plaster mixture [Figure 59] in the footprint mark and let it dry for about 15 minutes.

Figure 59 - Placement of the plaster mixture in the footprints

3. Carefully remove plaster cast (Evidence#11) by removing some soil around them (to facilitate the removal of casts) and wrap them in paper [Figure 60].

Figure 60 - Casts wrapped in paper

4. Allow the casts to solidify for one day.

5. Wash the plaster casts with running water and an old toothbrush [Figure 61].

Figure 61 - Washing of the plaster casts with running water and a toothbrush

6. Allow to dry and discuss the distribution and conservation status of the species to which the footprint corresponds [Figure 62].

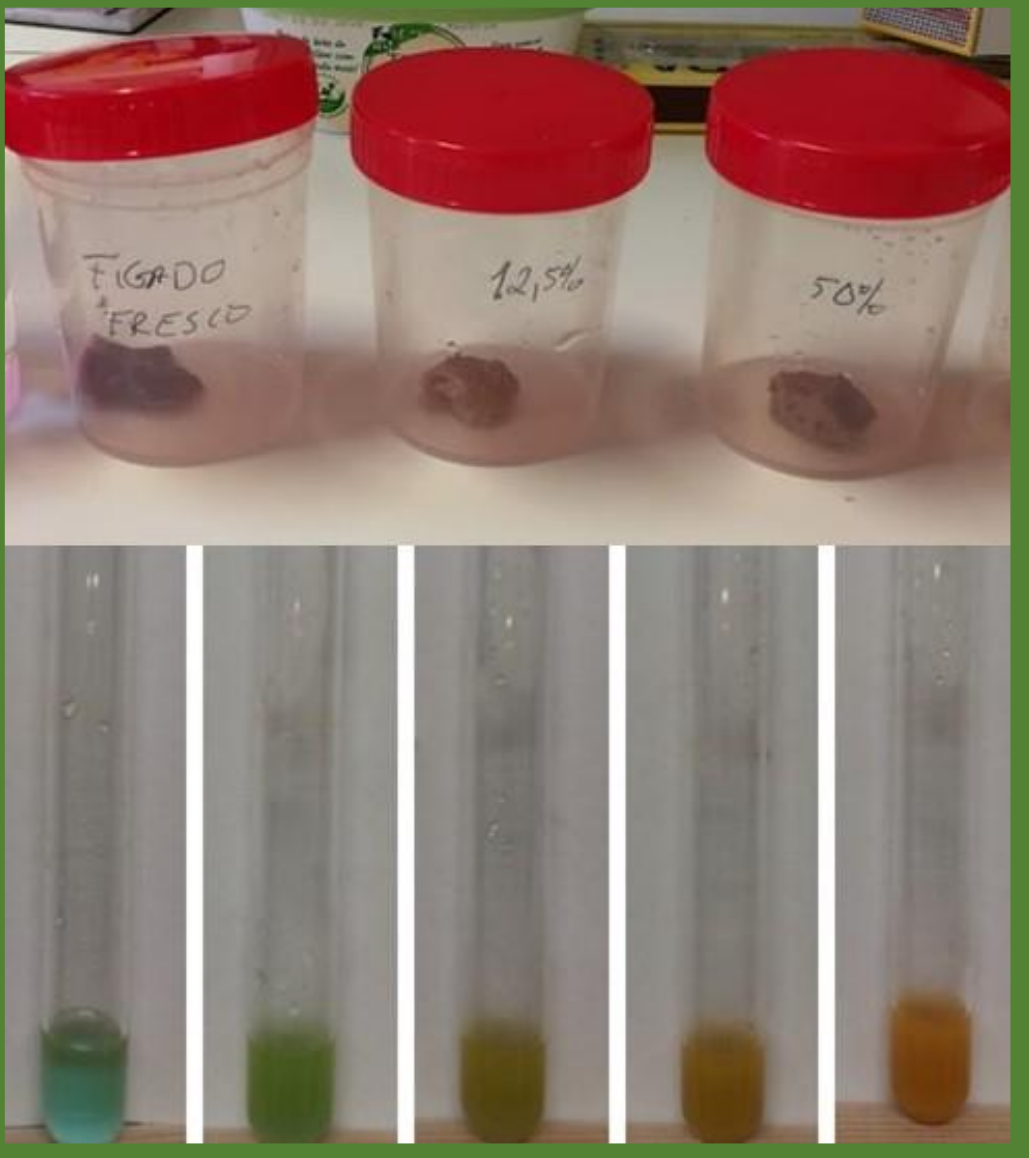
Figure 62 - Dry plaster cast

Questions and Answers

1. Do you think that in the hair samples observed, it would be possible to perform nuclear DNA analysis on the hair? Justify.

This answer will have to do with each hair observed, and it is up to the teacher to guide these conclusions based on the information

CHAPTER 3: *HEALTH EDUCATION*



Alcohol effect on liver

Biology

Objectives

With this chapter you will be able to:

- State the possible health consequences of excess of Alcohol consumption.
- Explain the general function of the liver in Alcohol metabolization.

Chemically, an alcohol is any organic compound where a hydroxyl group (-OH) is attached to a saturated carbon atom.

Ethanol

Ethanol is one of the main alcoholic components in alcoholic drinks. It is a volatile, flammable and colourless compound, mostly found in its liquid form. It has a somewhat sweet, characteristic odour.

Its chemical formula is C_2H_6O and can be produced by natural processes, predominantly by the fermentation method, present in yeast, or artificially, by petrochemical processes. Ethanol is widely regarded as a psychoactive drug, causing a characteristic intoxication and neurotoxicity when ingested in sufficient doses.

Potential harm of ethanol consumption

One of the most common drugs in our modern society, the harmful effects of ethanol and alcohol have been widely studied. These effects include: loss of balance, gastrointestinal disorders, short-term toxic allergic responses, damage in nervous, respiratory and renal systems, hormonal imbalances and cancer. Perhaps one of the largest known effects is the damage to the liver, which in extreme cases can be fatal.

Effect of Alcohol in Human Liver

The liver is the largest solid organ, the largest gland, and the most complex in the human body. In normal conditions, in addition to the metabolism of the ingested nutrients, bile production, production of essential substances to the organism and destruction of bacteria, **this is where the metabolism of toxic substances in the body occurs.** It is also an organ with great capacity for regeneration.

The liver is also the organ most affected by alcohol consumption as it out all the excess ethanol that is consumed by the body. Considered a toxin, this organ metabolizes alcohol by a series of "redox" reactions.

Forensic relevance

In forensic investigations, pathologists are familiar with situations of death in alcohol abusers, where the cause of death cannot be determined. In these cases, it is important to determine whether or not the death was related to the excessive consumption of alcohol.

It can be very difficult to determine the cause of death. Even in cases of severe alcohol abuse with a history of high prior consumption there may be little, or no alcohol found in the bloodstream after death. There may be no signs of violence or other drugs being involved. At autopsy, the only clue may be the presence of a fatty and enlarged liver. The pathologist must now study the liver closer to determine whether alcohol had a role in the death – looking at the cells and metabolites present in blood tests becomes important as they seek to draw conclusions as to the cause of death.

Excess of sugar consumption

Biology

Objectives

With this chapter you will be able to:

- Recall the possible health problems of excess of sugar consumption.
- Differentiate between “good” and “bad” sugars.
- State the basic molecular composition of different sugars.

Sugars are soluble carbohydrates. They may be monosaccharides, or simple sugars, such as Glucose, Fructose, and Galactose. Monosaccharides establish glycosidic bonds to form disaccharides, such as Sucrose (Fructose + Glucose), Lactose, and Maltose.

Glucose plays a key role in the metabolism of living beings, being the main source of energy for the cells. It is also a very versatile precursor in biosynthetic reactions.

Fructose, present in various fruits, in glucose syrup, and obtained in the hydrolysis of sucrose can enter into glycolysis after being converted into a phosphorylated derivative (3 glyceraldehyde phosphate), and its metabolism in humans occurs mostly in the liver and is not regulated by insulin, nor does it cause insulin release.

The blood glucose level is regulated by several organs, with the liver the largest metabolic regulator. In addition to the liver, the kidneys also play an important role in regulating blood glucose levels and are responsible for the filtration and reabsorption of blood glucose.

Muscles can rapidly consume glucose, avoiding blood glucose spikes.

In adipose tissue, fatty acids in the form of triacylglycerol are synthesized and stored from glycerol phosphate and free fatty acids, to be used as a substrate for gluconeogenesis (glucose formation) in the liver, when necessary. Adipose cells also produce Leptin, a hormone that controls energy balance, decreases appetite, and, consequently, induces weight loss.

Finally, the pancreas produces hormones such as insulin and glucagon, which are essential for the regulation of blood glucose levels.

Glucagon is released in response to low blood glucose levels, and has the function of maintaining stable plasma glucose levels between meals by stimulating gluconeogenesis, and the release of glucose by the liver, and inhibiting the glycolysis and synthesis of fatty acids. Thus, this hormone acts only in the liver. Its release is inhibited by insulin.

Insulin is released when blood glucose increases, and acts as a signal that cells with receptors for this hormone, such as those in the liver, muscle, and adipose tissue, increase their permeability to glucose, allowing it to enter to the inside. However, elevated insulin levels lead to desensitization of membrane receptors, leading to resistance to Insulin, which characterizes type II diabetes.

Consumption of sugars has been increasing over the years particularly due to the addition of sugars, such as sucrose and glucose syrup, in food and beverages (for example in soft drinks).

This excessive consumption can cause various health problems, such as obesity, type II diabetes type, metabolic syndrome, non-alcoholic steatohepatitis, and cavities.

The World Health Organization (WHO) defines obesity as "abnormal or excessive accumulation of fat that can cause health damage."

Obesity and being overweight increase the risk of cardiovascular disease, diabetes, musculoskeletal diseases (especially osteoarthritis), and even some cancers (cancer of the endometrium, ovary, breast, prostate, liver, gallbladder, kidney, and colon).

Type II diabetes causes an increase in blood glucose level due to insulin resistance. Initially, the pancreas produces an excessive amount of insulin to compensate for this resistance, but over time, it loses the ability to produce insulin, enough to keep blood glucose levels within normal range.

Symptoms of type II Diabetes include the urge to urinate more frequently, especially at night, thirst, excessive tiredness, unregulated weight loss, vision problems, among others.

Metabolic syndrome is the combination of diabetes, high blood pressure, and obesity.

Non-alcoholic steatohepatitis is a set of problems caused by an accumulation of fat in the liver, which can lead to serious damage to the liver, and even cirrhosis.

Studies indicate that the effects of fructose present as an additive in many foods and drinks lead to a worsening of these diseases. This is because fructose is converted to glycerol, which causes even greater weight gain than glucose, as well as decreasing Insulin and Leptin.

Recent research studies also suggest that a diet with too much sugar can also reduce the brain's ability to recall information as well as how well it learns from new experiences.

Forensic relevance

Diabetic Ketoacidosis, known as the "silent death" is a potentially life-threatening complication of diabetes. Symptoms may occur very rapidly – in some cases people may not realise that they previously had diabetes. When there is no apparent cause of death, pathologists can determine if Diabetic ketoacidosis (DKA) was responsible.

References

Alcohol effect on liver

Books and Journals

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- “Metabolismo do Álcool”, http://www.cisa.org.br/artigo/5536/metabolismo-alcool.php

Excess of sugar consumption

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ACTIVITIES

Activity X: Alcohol effect on liver

Objective:

To evaluate the effect of ethanol on animal cells.

To explain the metabolism of alcohol.

To explain the importance of Catalase in the metabolism of Hydrogen Peroxide

Curriculum Links

Key Stage 3:

Biology

Key Stage 4:

Biology, Chemistry

Time required:

Preparation of liver samples: 15 mins

Preparation of Ethanol Solutions: 15 mins

Liver exposure to Ethanol: 48 Hours

Catalase Activity in Hydrogen Peroxide:15 mins

Core Practicals / RPs/ PAGs

AQA – RP3 Biology
AQA – RP5 Chemistry
Edexcel – 1.16 Biology
Edexcel – 7.1 Chemistry
OCR – PAG B3 and C8

Safety Precautions:

- Wear gloves during activity.
- Be careful when using sharp objects.
- After using the materials, these should be sterilized with the use of suitable products.

Background Information:

Alcohol is one of the substances that affects the liver the most, since it is where most of alcohol metabolism occurs. In cases of alcohol abuse, it's associated the presence of a fatty and increased liver, and in more severe cases, the presence of scar tissue in the liver (cirrhosis that leaves it with a harder consistency) [Figure 63] that ultimately can cause liver cancer.

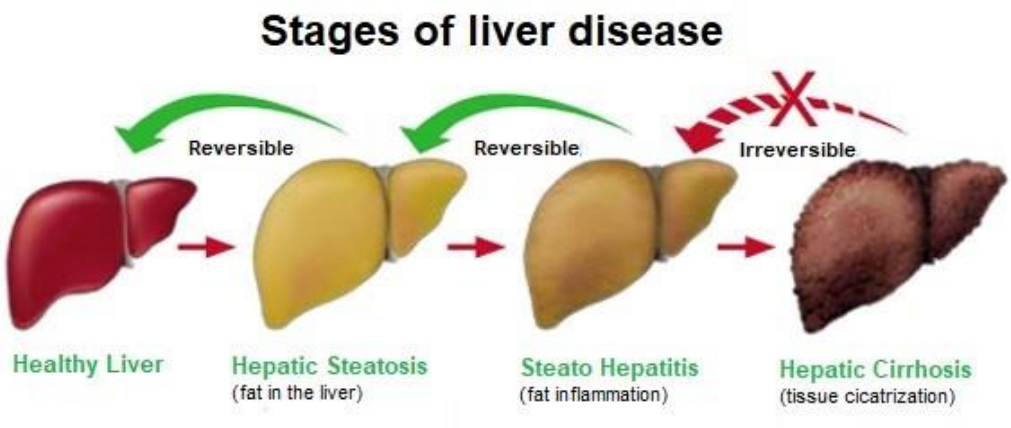


Figure 63 – Stages of Liver Disease

The main pathway of metabolization of alcohol [Figure 64] occurs through the enzyme alcohol dehydrogenase (ADH) which converts it to acetaldehyde. This is toxic to the body, even in small concentrations, and therefore needs to be converted to acetate by the enzyme aldehyde dehydrogenase (ALDH). This acetate will then be used in other metabolic processes in the body.

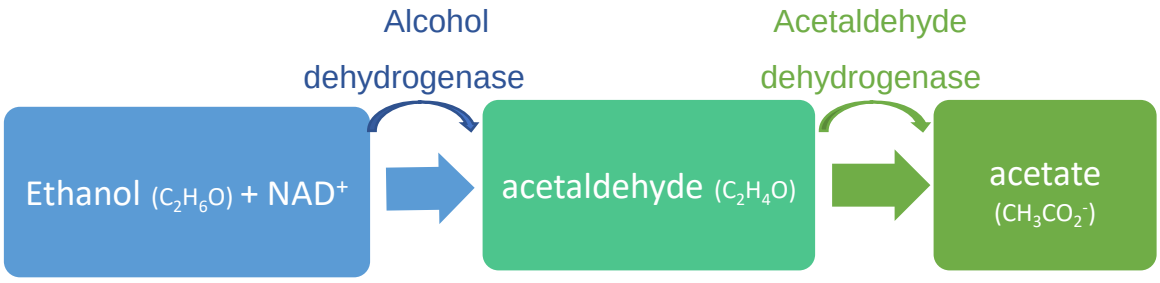


Figure 64 - Scheme of the main pathway of alcohol metabolism

Hydrogen Peroxide is a by-product of several important processes in the body, such as the immune system where it is produced as a defence mechanism against harmful organisms. It is also produced in the degradation of lipids and amino acids in the body. However, hydrogen peroxide is also toxic to the body's cells and needs to be degraded itself. This is done by the Catalase enzyme which is found in the peroxisomes in the cytoplasm of the cells. This enzyme degrades Hydrogen Peroxide into Water and Oxygen [Figure 65] molecules that can be used positively by the body.

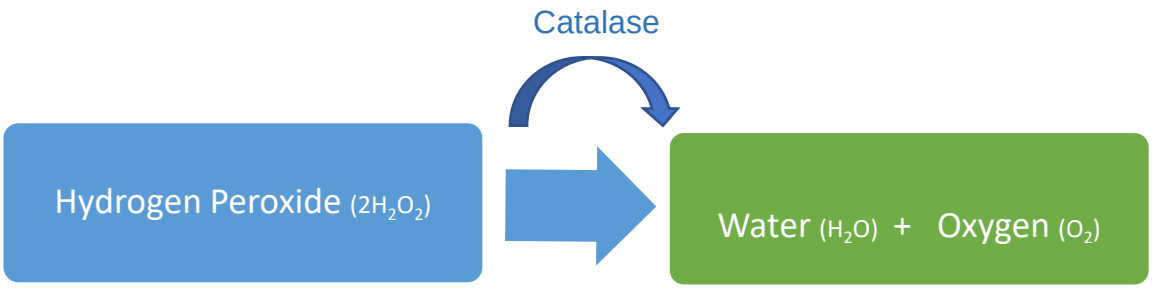


Figure 65 - Metabolization of Hydrogen Peroxide by Catalase

In cases where the physiology of cells is affected by excess consumption of alcohol, the metabolism of the liver is affected. This reduces the ability of the liver to change these toxic products into something less harmful and there is a build up of toxic molecules within the body.

Method to determine the effect of alcohol on the liver

Materials you provide

- Fresh liver (Evidence#8)
- Petri dish
- Digital scale
- 100ml plastic cylinder
- 2 - 100mL beaker
- 4 - 100mL cups with lid
- 6 boiling tubes with bungs
- Pipette filler
- 25ml graduated glass pipette
- Scalpel
- Ruler
- Ethanol (96%)
- Distilled water
- Hydrogen peroxide (5 Vol)
- Permanent marker
- Tweezers
- White tiles
- Gloves

Notes for Teachers

Notes for Technicians

Please note the timings needed for the following activities – and the need for the liver samples to be left at least 48 hours within the different concentrations of ethanol before subsequent analysis by students.

In procedure C the liver cup with detergent serves for students to compare the reaction of the flame in bubbles created only by the detergent (i.e. without oxygen as the reaction product) and with the oxygen bubbles resulting from the reaction.

Evidence#8 will correspond to the fresh liver used to the experience.

A. Liver Preparation

1. With the marker, identify the boiling tubes with numbers 1 to 6 (which correspond to the data recorded in the notebook with the weight and the respective measurements of the samples)
2. Place the Petri dish on the scale (tare to 0). On a white tile cut 6 pieces of liver (Evidence#8) with a mass of about 2g each.

3. Measure and weigh each liver sample accurately and write down the values. As samples are measured / weighed, each one is placed into each already numbered boiling tube from 1 to 6.

NOTES: Store a piece of liver in the refrigerator for 48H which can then be used for the rest of the activity.

B. Ethanol solutions preparation

1. Under numbers 1 through 6 of each boiling tube, note the respective ethanol concentrations. (0%, 12.5%, 25%, 50%, 75%, 96%);
2. In the numbered boiling tubes, with the liver samples (Evidence#8) inside, prepare each ethanol concentration to a total volume of 25ml according to Table 11.

Notes: To avoid the minimum evaporation of ethanol, first put the distilled water into each glass and only then the ethanol.

3. First place about 70ml of distilled water in the **100ml beaker** (to avoid contamination of the original water source) and in each corresponding cup, pipette the water values indicated in the table.
4. Then place in the other **100ml beaker**, about 70ml of Ethanol (96%) (to avoid containment of the original Ethanol source) and in each corresponding boiling tube, pipette the amounts of Ethanol indicated in the table and *cover immediately* with the lid to avoid evaporation of the blend. Shake each tube slightly to mix.
5. Leave the samples in the boiling tubes for **at least 48 hours**.
6. After 48 hours, remove each liver sample (Evidence#8) and note the respective weights and measures for comparison with the initial notes.
7. Put back each sample in the corresponding labelled boiling tube, having discarded the liquid from each. These samples will be used in the following activity.

Table 11 - Ethanol 96% Dilutions

Dilutions			
Cup Number	**% Ethanol**	**Distilled water quantities**	**Absolute Ethanol quantities (96%)**
1	0%	25ml	0ml
2	12,50%	22ml	3ml
3	25%	18.5ml	6.5ml
4	50%	12.5ml	12.5ml
5	75%	5.5ml	19.5ml
6	96%	0ml	25ml

C. Catalase Activity on the Hydrogen Peroxide

1. Carefully cut 2 pieces of fresh liver, each about 2g, and weigh on a petri dish placed on a scale (tared to 0). Make a note of the reading.
2. Place a liver sample in one of the four cups. Identify the cup as "Fresh Liver"
3. In a second cup place the other liver sample with a little water with detergent and identify the cup as "Liver with detergent".
4. Select from the previous experiment the cups with liver which had been exposed to concentrations of 12.5% and 50% of ethanol – place these pieces of liver in the remaining two cups.
5. Using the plastic cylinder put 15ml of hydrogen peroxide in each of the four cups, and immediately put a lid over each, so no gas may escape.
6. Allow the reaction to occur for 3 minutes. Observe the formation of the reaction product in each cup.
7. Meanwhile, stir well with a spoon the cup containing the detergent to form as many bubbles as possible.
8. After the 3 minutes, remove the lid from each cup in turn, and using a lit spill, observe the **initial reaction** of the flame in each of the four cups.

Notes for Teachers

You may have sufficient oxygen produced to relight a glowing spill (would be worth a check- almost certainly with the fresh liver) – but an already lit flame should burn visibly stronger in the presence of oxygen.

To measure the amount of oxygen given off by the different liver samples, set up a gas syringe and have students measure the volume of gas produced at 10-second intervals. Repeat for different samples and plot graph of results. This links in well with the Required Practicals for Rates of Reaction:

Questions and Answers

1. Review all your measurements and the observations you have made including the physical appearance of the liver when exposed to difference concentrations of ethanol. What conclusions can you make about the impact on the liver's ability to metabolize toxic substances in the body?

When the liver is exposed to increasingly high concentrations of ethanol, it becomes drier (alcohol causes dehydration) and with a more rigid consistency, which leads to the conclusion that there will also be changes at the cellular level and consequently the metabolic capacity of the liver will also be affected.

2. What are the bubbles that form after hydrogen peroxide is added to the liver? How did you determine this?

The bubbles formed are oxygen which is one of the products of the Catalase reaction (present in liver cells) with hydrogen peroxide. Oxygen was not formed in the bubbles with liver and detergent. The flame increased in size when in the presence of oxygen.

3. What difference did you notice in the production of bubbles / oxygen relatively to the increasing percentage of Ethanol to which the liver was exposed. Explain your answer

The higher the percentage of ethanol to which the liver was exposed, the fewer bubbles were produced and the flame did not burn as bright. This is because less oxygen was produced in the reaction.

This is because the greater the percentage of ethanol the liver was exposed to, the greater the damage to the liver – this was also seen in the changes to its appearance. This damage meant the liver was less able to break down the hydrogen peroxide into oxygen and water.

Activity XI: Sugar in Blood

Objective:

To state the differences between Sucrose and Fructose, and Simple and Complex Sugars.

Curriculum Links:

Key Stage 3 and 4:

Biology and Chemistry

Key Stage 5:

Biology

Required time: 90 minutes

Core Practicals / RPs/ PAGs

AQA RP4 Biology
Edexcel – 1.13B
OCR – PAG B8

Safety Precautions:

- Wear gloves and protective clothing
- Benedict's reagent is an irritant – wear eye protection
- The heating plate requires special care as it reaches high temperatures.

Background Information:

Carbohydrates, also called sugars, are one of the components of our diet, but they do not all have the same effect on the body. The metabolism of sugars in the body depends on its degree of polymerization, i.e. simple sugars are more easily metabolized than complex molecules. Therefore, the consumption of complex sugars is more beneficial to the body, unlike simple sugars, which have more harmful effects on health.

Carbohydrates are polyhydroxylated aldehydes, polyhydroxylated ketones or compounds which, upon hydrolysis, can be converted. These compounds may be divided into monosaccharides, disaccharides and polysaccharides, according to their degree of polymerization.

Monosaccharides are so-called simple sugars and therefore can no longer be hydrolysed. They are generally crystalline, sweet and water soluble, of the general structural formula $[CH_2O]$ n, with the carbon number being greater than

2 (n> 2). In addition, they are characterized as reducing sugars, that is, their aldehyde and ketone groups can undergo oxidation, and are classified according to their number of carbon atoms. The most common and crucial functions in living beings are the aldo-pentoses and the aldo-hexoses, which respectively have 5 and 6 carbon atoms.

The hexoses that stand out for their importance are glucose and fructose, which obey the general formula $C_6H_{12}O_6$ and are the main energy sources of living beings, since they are energy-rich biomolecules, constituting the main cellular fuels. The most abundant monosaccharide in the body is glucose, whose chemical structure is represented in Figure 66 (A). In metabolism, glucose is one of the main sources of energy and provides 4 calories of energy per gram. In relation to fructose, whose chemical structure is illustrated in Figure 66 (B), this is known as the fruit sugar and is characterized by being sweeter than the sucrose itself.

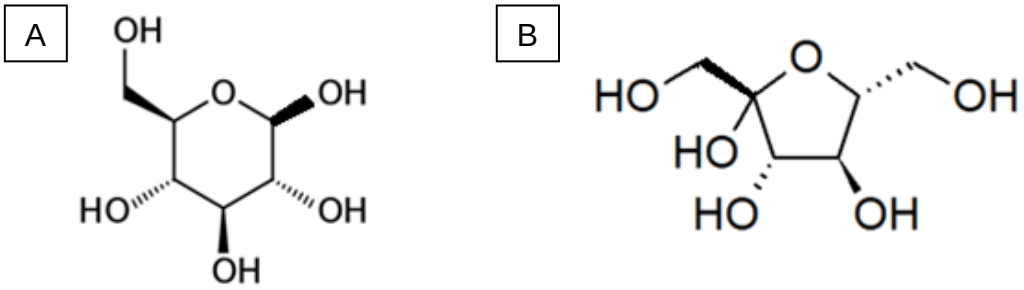


Figure 66 - (A) Glucose structure (B) Fructose structure

Fructose participates in the glycolytic pathway, so if excessive amounts of this sugar are ingested, this pathway will be overloaded, leading to the formation of high amounts of acetyl-CoA, which increases the biosynthesis of fatty acids, causing accumulation of fat in adipose tissue.

Disaccharides are characterized in that they can be hydrolysed into two monosaccharide molecules, for example sucrose, which can be hydrolysed to glucose and fructose, as can be observed in Figure 67.

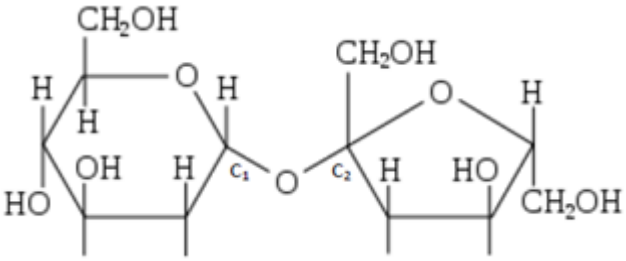


Figure 67 - Structure of sucrose

They are relatively small molecules and soluble in water, which can alter the osmotic balance of the cells, and are also the main form of transport within the carbohydrate group. The most important and common disaccharides besides sucrose are maltose and lactose. The sucrose, shown in Figure 67, assembles glucose and fructose through a glycosidic bond between carbons 1 and 2.

The oligosaccharides have from 3 to 9 degrees of polymerization (from 3 to 9 monosaccharide molecules), while the polysaccharides have a degree of polymerization of 10 or more (from 10 molecules / monosaccharides). The latter are the complex sugars or macromolecules, and can be hydrolysed in several molecules of monosaccharides, through the action of certain enzymes. Starch, glycogen and cellulose are among the most known examples of this type of carbohydrate.

What happens?

In order to understand the chemical reactions involved in the presence of carbohydrates, a colour scale will be made.

The reducing sugars are involved in a chemical reaction which indicates the presence of carbohydrates. Benedict reagent contains copper (II) ions which are reduced by the reducing sugars, forming reduced copper (I) ions which precipitate in the form of copper oxide. However, this chemical reaction, which is represented in Figure 68, only happens in the presence of high temperatures.

Figure 68 - Chemical reaction that allows to detect the presence of reducing sugars, based on the Benedict Reagent

The sugar reducing group will reduce the copper ions present in the Benedict reagent in the presence of heat, thereby forming copper oxide, which has a characteristic reddish brown or “brick red” colour. Observing this reaction, you will see the original blue colour of the copper ions in the alkaline solution, change to the “brick red” colour.

The intensity of the colour produced depends on the concentration of the sugar in the solution. Testing a range of concentration of sugar solutions allows a colour scale to be produced. *Note: the colour observed may not be the expected “red-brick” colour – the key issue is to observe the colour change, and the intensity of the colour produced.*

Rationale for sugar-products being tested

Sugar appears in many different products, some of which are popular amongst children. With the growing concern about childhood obesity, the following products have been chosen for comparison:

- Fruits (apple, pear)
- Drinks (fruit juice and lemonade)
- Sweets (e.g. polos and white mini-marshmallows)

Sweets, often popular with children, contain a large amount of simple sugars, which are rapidly absorbed and can cause problems in the body when consumed in excess. Soft drinks are also very popular among children, and they also have considerable amounts of sugar.

Method to determine the amount of sugar present

Material in the Toolbox

- Pasteur pipette
- Fructose
- Benedict's Reagent
- Gums

Materials you provide

- Waterbath
- 17 Test tubes
- Fruit, Sweets, lemonade, fruit juice

Notes for Technicians

When choosing the soft drink and sweets to test, select those of a pale or transparent colour to facilitate observing the strength of colour produced.

Part 1: Colour Scale

Make the colour scale:

1. Prepare test tubes with various solutions of increasing concentrations of fructose:
   a) Add fructose (0g, 0.05g, 2,5g, 10g) to 10 ml of water and mix thoroughly (use vortex if necessary).

   Caution: Make each concentration in a different tube.

   b) Using a Pasteur pipette add 30 drops of each solution into each test tube.
   c) Label each test-tube with the amount of fructose added
2. Add 10 drops Benedict's Reagent to each test tube
3. Prepare a water bath to a high temperature
4. Place the test tubes in the water bath for 1min 30secs
5. Remove the test tubes and place in a rack. Allow to cool for 2 minutes
6. Order the test tubes by concentration to build your own colour scale

Part 2: Sugar Quantification

1. Prepare the test tubes with the different sugars being tested. Use 1 g of each of the solids (ground up) and 10 drops of the liquids. Label the test tubes.
2. Using a Pasteur pipette, add 30 drops of water to each test tube.
3. Add 10 drops of Benedict's Reagent to each test tube
4. Place the test tube in a water bath for about 3 min
5. Place the test-tubes in a rack to cool for 2 minutes
6. Compare the results with the colour scale below, as well as the one you made in Part I. [Figure 69].

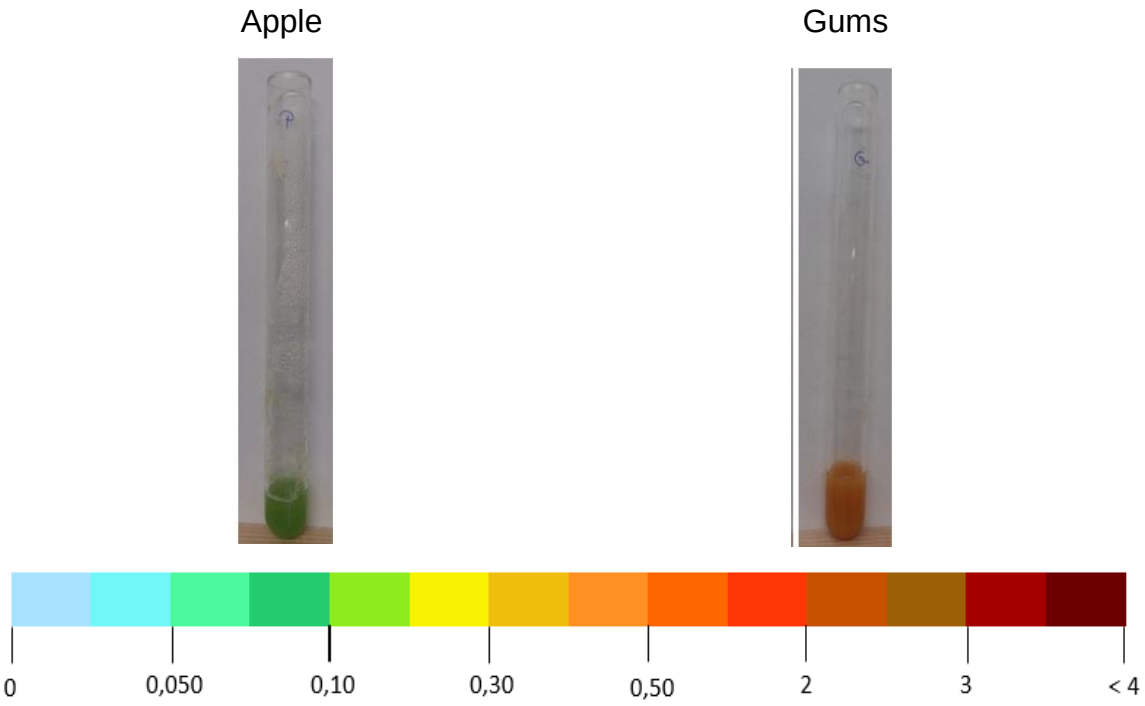


Figure 69 - Colour scale, according to the different amounts of fructose (in grams)

Questions and Answers:

1. Which are the benefits of sugar?

   It is the main source of energy in vascular plants and animal cells.

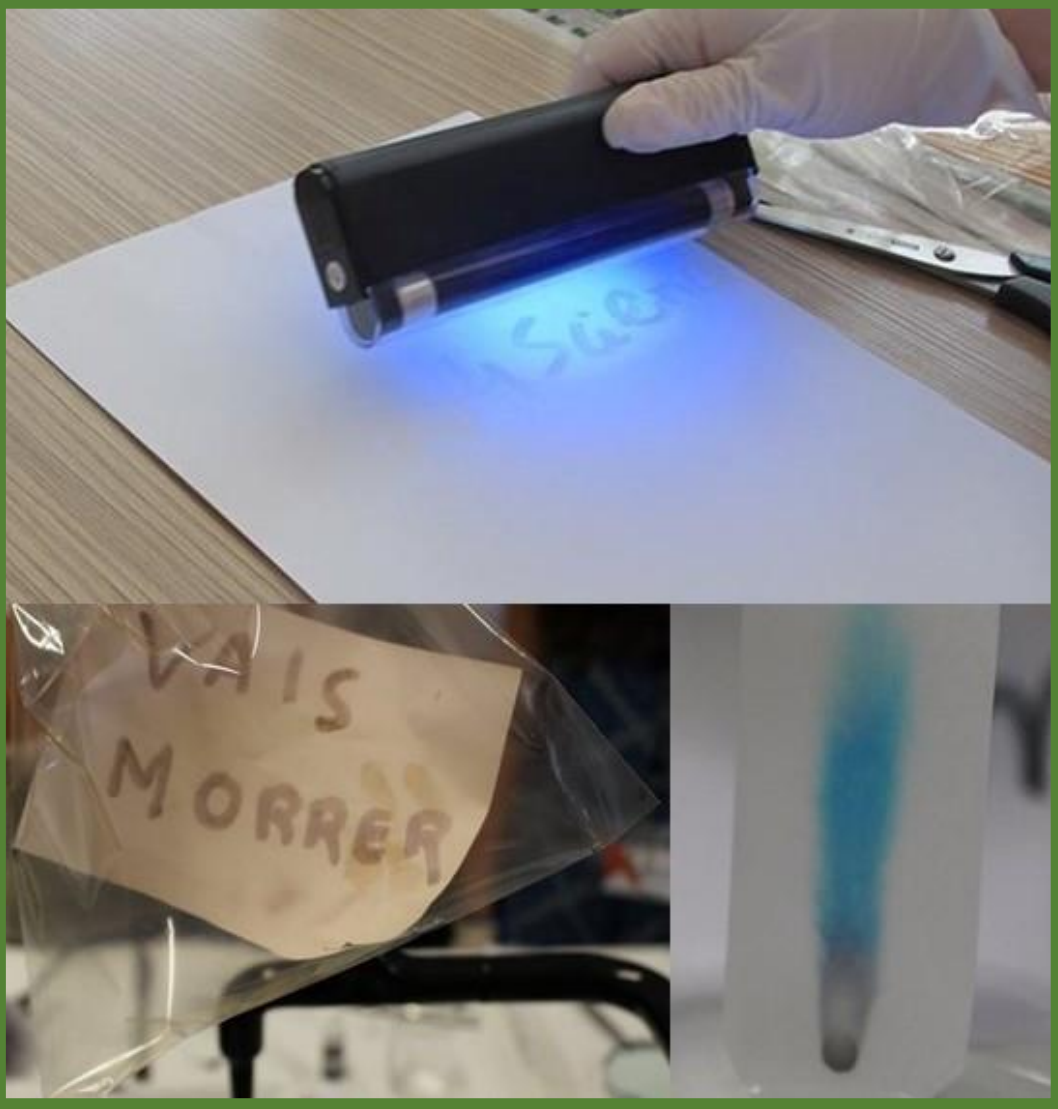
2. Fructose is a:

   a) **Oligosaccharide**
   b) **Polysaccharide**
   c) **Monosaccharide**

3. Which are the consequences of excessive sugar consumption?

   For example: Diabetes, Metabolic Syndrome, Obesity, Cavities.

Chapter 4: CHEMISTRY



Document Evidence

Chemistry

Objectives

With this chapter you will be able to:

- Describe what a questioned document is
- Identify the document analysis methods

Documents are increasingly being viewed electronically on computers and are moved around in cyberspace. But documents continue to exist in paper form and are a well-known form of evidence.

Document analysis, a very broad area in the field of forensics, is the examination and comparison of questioned documents with known material. A questioned document is an original, valid document that has been changed in some way and can be any signature, handwriting, typewriting, or other written mark. Examples of questioned documents include forged cheques, certificates, passports, licenses, money, letters, contracts, disputed wills, suicide notes, altered receipts, and even altered lottery tickets.

The polymeric materials which can be analysed on documents are ink and paper.

Ink

Ink is one of the most common materials used in the production of a document. There are a number of different types of writing instruments each using a particular kind of ink. The most common are pen inks, certain ballpoint (biro) pen inks. Other types, such as gel pen inks, are becoming more popular.

Analysis of inks used in questioned documents can provide various types of useful information. The analysis can determine if two inks are of the same origin, if they were made by the same manufacturer or even when the ink was manufactured.

Ink Composition

Ink is a complex medium and can include colourants (dyes and pigments), solvents and resins – which 'carry' the colourant and are therefore known as the vehicle. There are also other organic and inorganic ingredients that may be present, which can include antioxidants, preservatives, wetting agents, lubricants, and trace elements, although these typically form a small fraction of the overall formulation.

Colourants are a crucial part of all inks. Two types of colorants can be used: dyes and/or pigments. The major distinguishing feature between dyes and pigments is that pigments consist of fine particles of insoluble material that are suspended in the vehicle. Pigments are generally considered more stable and long-lasting than dyes because pigments are less prone to photodecomposition (they are lightfast) and are insoluble in water. Their colour can be derived from a metal-centred complex and are generally less vibrant than dyestuffs. Additionally, pigments are more opaque than dyes, so the colourant is more efficient at masking any underlying material.

The fluid portion of ink that suspends and delivers the colorant to the substrate is known as the vehicle. Vehicles are necessary to carry the colour from the cartridge to the paper. Once on the paper, the solvent undergoes a series of changes over a fixed period of time, causing the colourant to dry onto the paper. The resins, which can be natural or synthetic, are polymers that are incorporated into inks to provide them with a desired viscosity and a means to bond the ink and the paper as the ink dries. Normally, the resinous material is dissolved into the vehicle to create a solution in which colourants can be added.

Paper

Currently, the majority of documents are produced on traditional paper and, consequently, paper analysis is an important part of the work undertaken by forensic document examiners. Although much talked about, the 'paperless office', where most, or all, information is stored and communicated electronically, has become a reality only in a few specialist areas. The use of paper still represents the preferred option for many types of documentation.

Paper is usually made from plant material, with the main constituent being cellulose fibres and wood being a major source of the fibres. During paper manufacturing, sizing agents are usually added to make the cellulose component more hydrophobic (molecules repel from contact with water) to prevent ink from running. An array of minerals, resin and colorants can be present in paper and as the composition varies between manufacturers, paper chemistry can be used to connect a paper specimen to a source.

Document analysis methods

Forensic document examiners use several methods to determinate the validity of a questioned document. The first step is always to examine the document with the naked eye. A surprisingly large percentage of forgeries are so crudely done that the forgery is obvious even through quick examination. If anything untoward were detected in the document, there are other tools available, such as: examination with alternate light sources, chemical analysis and microscopic analysis.

Alternative light sources

Papers and inks that are indistinguishable under white light may have very different appearances under ultraviolet (UV) or infrared (IR) wavelengths. Some erasures and other modification that would look normal under white light may stand out under UV or IR illumination. The infrared light detects different inks and dyes. Infrared wavelengths make scratched out or erased words easy to read. Ultraviolet lights make oils and chemicals visible.

Examination with an alternative light source (ALS) is normally the first step in any document analysis. Often, no other steps are needed to reveal alterations.

Chemical analysis

Analysis of the chemical composition of documents provides information about the origins and whether or not modifications have been made. Forensic document examiners use numerous chemical tests to compare inks and paper.

An analysis of the chemical composition of writing ink present on documents may verify whether known and questioned documents were prepared by the same pen. One of the most widely used and generally accepted methodologies employed to compare and help characterize ink formulations is Thin-Layer Chromatography (TLC). TLC is an effective and efficient method for separating and identifying colorants [Figure 70]. Most commercial inks, especially ballpoint inks, are actually mixtures of several organic dyes. The separation pattern of the component dyes is distinctly different for inks with different dye compositions and thus provides many points of comparison between a known and a questioned ink.

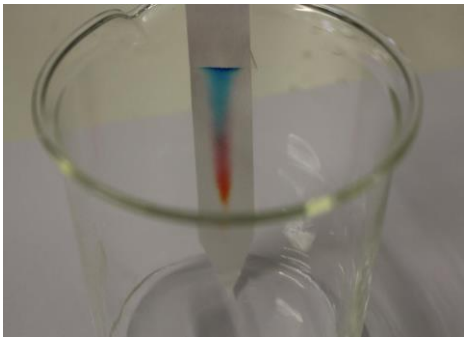


Figure 70 - Example of a thin-layer chromatography

Microscopic analysis

In microscopic analysis, handwriting analysis is the main component. A visual examination of the writing on a document, using low-powered microscopy, may provide general information on the type of ink, and therefore the type of writing instrument used. Pen inks are either water-based or oil-based. Water-based inks are used in fountain pens and fibre-tipped pens, for example. Oil-based inks are used in ballpoint pens, which are the most frequently encountered type of pens in casework. The interaction between a water-based ink and the surface of a piece of paper differs from the interaction between an oil-based ink and paper. This is because the paper surface is fibrous and absorbent, and the fluid water-based ink will tend to run along the fibres on the paper surface, which does not occur with oil-based inks. Oil-based inks often appear to sit on the paper surface and have a shine to them, whereas water-based inks have more of a matt (non-shiny) appearance.

References

Document Evidence

Books

- Harold H. Trimm. Forensics the Easy Way. Barron's, 2005.
- Katherine M. Koppenhaver. Forensic Document Examination: Principles and Practice. Human Press, 2007.
- Lawrence F. Kobilinsky. Forensic Chemistry Handbook. Wiley, 2012.
- Michael Allen. Foundations of Forensic Document Analysis: Theory and Practice. Wiley Blackwell, 2016.

Web sites

- "Forensic Document Examination", http://www.forensicsciencesimplified.org/docs/how.html
- "What is Forensic Document Examination?", http://www.safde.org/whatwedo.htm

Real Life Case Studies

The Hitler Diaries (1983)

Figure 71 – Adolf Hitler

In April 1983, the news magazine *Stern* published passages from what they claimed to be the diaries of Adolf Hitler, a series of books written between 1932 and 1945. *Stern* journalist, Gerd Heidemann, claimed to have paid 10 million German marks for the 60 small books, which had allegedly been smuggled from a crash site in Dresden by 'Dr Fischer'.

One page was taken from the diaries and examined by handwriting experts in Europe and the USA, which resulted in numerous experts agreeing that the handwriting did in fact belong to Hitler. However, when a press conference was held, writer David Irving presented photocopies of another fake Hitler diary, claiming it was from the same source as the magazine's material. This sparked further controversy, so the diaries were further analysed. Bundesarchiv, the German Federal Archives, soon established that the diaries were written on modern paper with modern inks, proving them to be counterfeits. Furthermore, Dr Julius Grant in London conducted a forensic analysis of the diaries, agreeing that the diaries were in fact fakes.

It was discovered that the so-called Hitler diaries were actually written by Konrad Kajau, a notorious Stuttgart forger. Kajau and Heidemann were both sentenced to 42 months in prison.

ACTIVITIES

Activity XII: Invisible Ink

Objective:

Demonstrate how the acidic properties of lemons can be used to make lemon juice act as an invisible ink and how invisible messages can be seen with UV.

Time required: 30 minutes

Suggestion of curricular applicability:

Chemistry:

Key Stage 3 &4

Core Practicals / RPs/ PAGs

N/A

Safety Precautions:

- Wear protective gloves and clothes, safety goggles
- When using open flame source or a hot plate, take great care to avoid burns

Background Information:

Forensic document examination, also referred to as questioned document examination, is a branch of forensic science that includes the examination of paper, ink, typewriting, and writing instruments.

Ink analysis is an important part of the investigation of question documents; however, the ink may often be hidden (invisible), making it difficult to analyse it.

Secret or invisible ink is a substance made with many different substances and has been widely used in steganographic schemes (practice of concealing a file, message or image within another file, message or image) so that secret messages can be invisibly written on papers. Broadly, invisible inks can be categorized as: organic and sympathetic inks. Organic inks consist of “natural” methods, such as lemon juice, vinegar, milk, sweat, saliva and onion juice. Sympathetic inks are chemical solutions that can contain one or more chemicals.

Hidden messages written with invisible ink can be made visible by a revealing process depending on the type of invisible ink. The organic invisible inks can be revealed through heat, such as with fire, irons, or light bulbs, and some can be seen when placed under ultraviolet light. Sympathetic inks require the application of a specific chemical (called the reagent) to be developed, such as another chemical or a mixture of chemicals.

The search for characters secretly written in a document is a practice which comes from ancient times, such as the detection of espionage in wartime or communication with the outside world by prisoners.

How does it work?

Organic inks are the most used type of invisible ink and can be revealed through heat or ultraviolet light.

In the heat process, the organic inks alter the fibres of the paper (making them weaker) so that the secret writing has a lower burn temperature and turns brown faster than the surrounding paper when exposed to heat. Inks revealed by heat are usually acidic. They change the chemical composition of the paper and also oxidize when heated. This oxidation (loss of electrons) means they undergo a change in their chemical composition. It is this oxidation which turns the compounds brown and reveals the previously hidden ink.

An example of one such invisible ink is lemon juice, also known as citric acid. Lemon juice is composed of sugar, water, and citric acid. None of these components contain much colour, and therefore appear “invisible” after the lemon juice dries on paper. The citric acid also inhibits the oxidation of the other components, preventing browning. Chefs often add a splash of lemon juice to freshly peeled apples, to prevent oxidation and unsightly browning before they are cooked.

When used as an ink, the lemon juice can be made visible by the process of heating. This causes the citric acid to decompose which releases the carbon atoms previously held within the structure of the citric acid molecule [see Figure

72] . It is these carbon atoms which now become visible, showing as a brown colour.

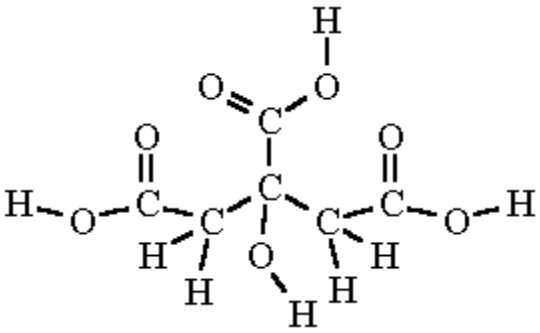


Figure 72 - Chemical structure of citric acid ($C_6H_8O_7$)

On the other hand, certain inks invisible to the naked eye become visible under an ultraviolet light (light in the range of 100nm to 400nm). Invisible inks contain substances that fluoresce when exposed to a UV light source. The material absorbs a portion of energy and emits fluorescence in the visible spectrum when excited with UV lighting. Many organic compounds do this, as do laundry detergents and sunscreens.

Sunscreen is made of inorganic (non-carbon based) and organic (carbon based) materials that fluoresce when exposed to a UV light. Inorganic materials include zinc oxide (the component that makes sunscreen white) and titanium dioxide. They physically block UV rays and reflect, scatter and absorb the UV light. On the other hand, organic materials act chemically, absorbing the UV light through their chemical bonds. As the bonds absorb UV radiation, the components of the sunscreen slowly break down and release heat.

Method to reveal invisible ink

Materials in the Toolbox

- Cotton Swabs
- Invisible Ink
- Evidence#6
- Gloves

Materials you provide

- Protective clothing
- Cotton Swabs
- White Paper
- Paper towels
- 50ml beaker
- 100ml Beaker
- Knife
- Alcohol or Bunsen burners or hot plate
- Fresh whole Lemons
- Sunscreen SPF 50
- Water
- UV light

Part 1: Analysis of unknown sample

1. Put on your gloves and protective clothing.
2. With a UV light, check if there is any message written in Evidence#6.

Notes for Teachers

During class, students can discuss why the message is visible with ultraviolet light and can also can make their own invisible ink using the following methods.

Sunscreen as invisible ink

1. Put on your gloves and protective clothing.
2. In the beaker, measure 10ml of sunscreen and 40ml of water and transfer the solution to the 100 ml beaker.
3. Gently swirl the solution to mix it – *you may need glass rod to assist this*
4. Dip the cotton swab in the sunscreen solution until the cotton is covered with sunscreen solution (your “ink”). Remove the excess solution by gently dabbing the swab on paper towel.

5. With the “ink” from the swab, write or draw something on the white paper. You may need to repeat step 3 several times to complete your message or picture.
6. Wait 5 minutes to allow the paper dry. Once dry, you'll have what looks like a blank sheet of paper.
7. Carefully hold your ‘blank’ piece of paper under a UV light source and observe your message become visible

Lemon juice as invisible ink

1. Put on your gloves and protective clothing.
1. With a knife, carefully cut a lemon in half and squeeze its juice into a 50mL beaker.
2. Dip the cotton swab in the lemon juice until the cotton is covered with juice (your “ink”). Remove the excess juice by gently dabbing the swab on paper towel.
3. With the “ink” from the swab, write or draw something on the white paper. You may need to repeat step 3 several times to complete your message or picture.
4. Wait 5 minutes to allow the paper to dry. Once dry, you'll have what looks like a blank sheet of paper.
5. Using tongs, hold the paper parallel to the heat source and move it so the flame passes just below the message. The message should appear immediately.

Caution: do not hold the paper too close to the flame, because it can easily ignite. Alternatively, use some tongs to place the paper on or above a hot plate. Remove the paper as soon as the message becomes visible.

Questions and Answers:

1. Indicate and explain the different types of invisible inks.

Invisible inks can be categorized as: organic and sympathetic. Organic inks consist of "natural" methods, such as lemon juice, vinegar, milk, sweat, saliva and onion juice. Sympathetic inks are chemical solutions that can contain one or more chemicals.

2. Indicate the three methods of revealing invisible ink.

Invisible ink can be revealed through heat, ultraviolet light and chemicals.

3. The UV light range is:

a) 100nm to 400nm
b) 400nm to 800nm
c) 800nm to 1mm
d) none of previous

4. Explain the role of the citric acid e.g. in preparing food such as apples.

The citric acid inhibits the oxidation of the other components (sugar and water) preventing the lemon from browning.

Activity XIII: The colour of Guilt – Chromatography

Objective:

To state the purpose of chromatography

Measure and graph pigment separation

Use chromatography to determine the colour composition of several inks.

Curriculum Links

Chemistry:

Key Stage 3 and 4

Core Practicals / RPs/ PAGs

AQA – RP12 CS – Chy
Edexcel – 2.11
OCR – PAG C3

Time required: 90 minutes

Safety Precautions:

- Wear protective gloves and clothes, safety glasses
- Ethanol is flammable –  handle with care

Background Information:

In the investigation of questioned documents, ink analysis is an important step. Since ink is used for writing, painting and drawing purposes, its analysis can give relevant information about the questioned document. The central aspect of ink examination is determining the formulation of the ink.

In ink analysis, the separation of its components is performed using the chromatography technique. Chromatography separates substances within a mixture based on their physical properties.

In chromatography, the components of a mixture are dissolved into a solvent (mobile phase) and the different components are separated according to how they interact with the material of the stationary phase. There are various separation processes which can be divided into three categories:

1. Thin layer chromatography (TLC) – also called paper chromatography, the stationary phase is a thin layer of material supported, for example, on a glass or aluminium plate;
2. Liquid chromatography – the solution containing the mixture is forced through a thin column packed with a stationary phase material;
3. Gas chromatography – the tested mixture is in gaseous form and passes through a narrow column the surface of which is covered with a stationary phase material.

The most common forensic method for analysing inks from documents is thin layer chromatography. TLC is a simple chromatographic technique used to separate non-volatile mixtures with a rapid response.

TLC works with the same principle as all chromatography techniques: a substance will have different affinities for the mobile and stationary phases, and this affects the speed at which it travels. The goal of TLC is to obtain well defined and separated spots (i.e. the ink splotch is separated into different dye components, which show up as differently coloured bands).

The result of the chromatography is called a chromatogram. A chromatogram shows how far the solvent travelled and the substances that were dissolved in the original mixture, forming a colour pattern [Figure 73].

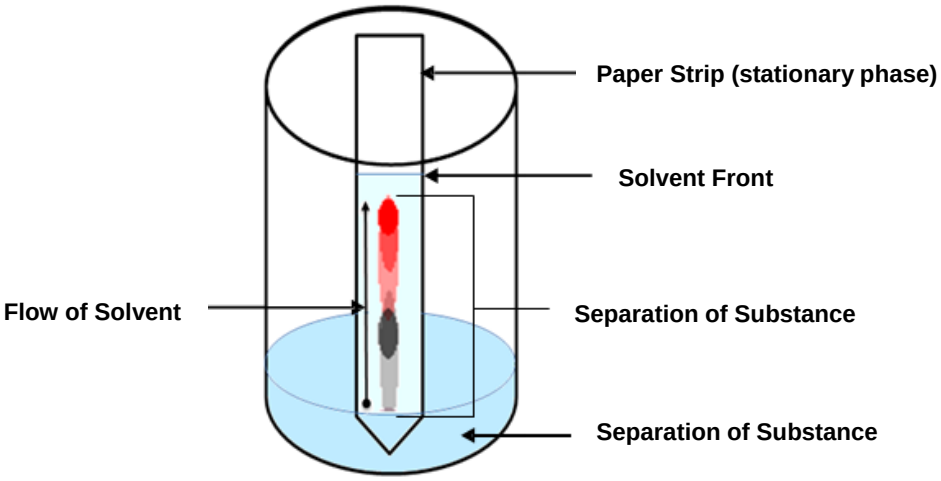


Figure 73 - Example of separating substances from an ink using TLC

How does it work?

In paper chromatography, a small amount of ink is removed from the document and placed onto a strip of an absorbing paper, which is then dipped in a solvent, such as water, alcohol or acetone. The solvent rises through the paper (stationary phase) by capillary action (the movement of the solvent on the paper due to the attraction of the molecules of solvent to the molecules in the paper). As the solvent moves up through the paper, it dissolves the ink mark into a mixture of its component parts. Substances in the mixture that are strongly attracted to the travel slowly and leave a coloured spot lower down the strip of paper. Substances less attracted to the paper spend more time dissolved in the solvent and travel more quickly up the paper.

The various substances in the mixture are therefore separated, leaving coloured spots at different levels on the paper. These substances can be identified by the ratio of the distance they travelled to the distance travelled by the solvent, called retardation factor (R_f) [Figure 74].

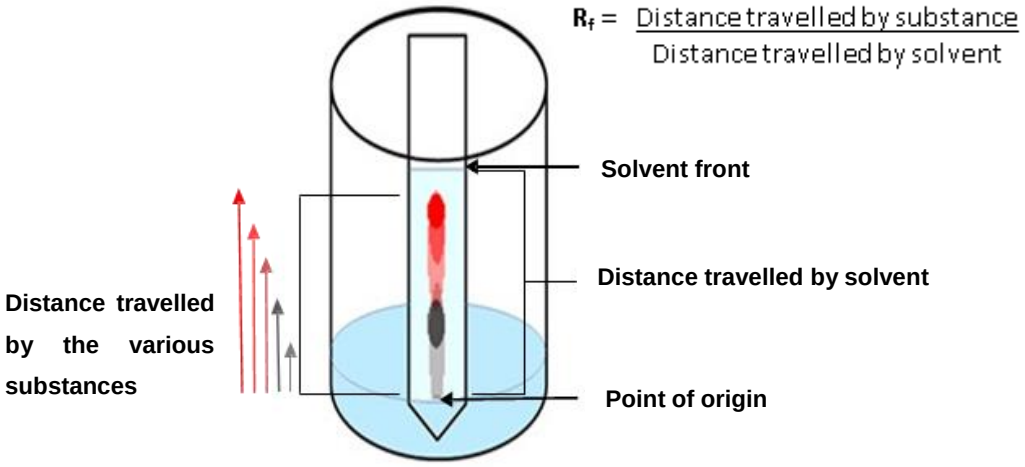


Figure 74 - Procedure for calculation of R_f value in paper chromatography

The actual distances travelled by the substances and the solvent may vary between experiments, but the R_f value remains constant for a particular substance. Therefore, identical substances have the same R_f values.

The Rf values strongly depend on the nature of the solvent. The R_f value is small when a substance is strongly attracted to the paper and does not travel very

far from the point of origin. The ideal solvent is the one that provides the best separation of the different substances contained within the mixture.

Method to separate dyes in inks

Materials in the Toolbox

- Chromatography paper
- Evidence#6
- Evidence#7
- Evidence#10
- Gloves

Materials you provide

- Protective clothing
- Pencil
- 600ml Beaker
- Ruler
- Scissors
- Adhesive tape
- Watch glass
- 96% Ethanol
- Distilled water

You can make your own chromatography paper using the following steps:

Notes for Technicians

a) Cut chromatography paper approximately 12cm long and 2cm wide. Use the paper chromatography provided in the Toolbox as template;

b) Cut one end of each strip into a point – see Figure 75.

Part 1: Separating pen ink using chromatography

1. Put on your gloves and protective clothing.
2. Using Evidence#7, draw a dot with 2-3mm in size at 2cm from the pointed end of the strip (this point will touch the solvent) [Figure 75]. Repeat the process for the Evidence#10 using another strip.

Figure 75 - Paper strip for separation test

3. Label your strips at the top with a pencil so you can tell them apart.

4. In the beaker, put 30ml of ethanol and 10ml of water
5. Put the strips inside the beaker and tape them with adhesive tape to the top of the beaker so they do not move [Figure 76]. The strip should be in contact with the solution, while keeping the dot above the solvent level and without touching the walls of the beaker.
6. Cover the beaker with a watch glass or some other object.

Figure 76 - Beaker with test strips

7. Wait 35 minutes to allow the solvent to soak up the strip.
8. After that time, remove the strips from the beaker and place a mark where the solvent reached. Then allow the filter paper to dry.
9. Record your work in Table 12.

Table 12 - Table of test results – example data

Substance	Evidence#7			Evidence#10		
	Distance travelled by substance (cm)	Distance travelled by solvent (cm)	R_f Value	Distance travelled by substance (cm)	Distance travelled by solvent (cm)	R_f Value
Substance 1	*0.6*	*6.3*	*0.095*	*0.3*	*6.4*	*0.047*
Substance 2	*1.2*	*6.3*	*0.190*	*0.9*	*6.4*	*0.141*
Substance 3	*4.5*	*6.3*	*0.714*	*3.9*	*6.4*	*0.609*
Substance 4	*6.3*	*6.3*	*1*	*6.4*	*6.4*	*1*

Part 2: Examining documentary evidence with chromatography

1. Put on your gloves and protective clothing.
2. Using Evidence#6, put 4 drops of water on the last 3 letters of the victim's name and with paper towel, gently remove the excess water without touching the ink [Figure 77].

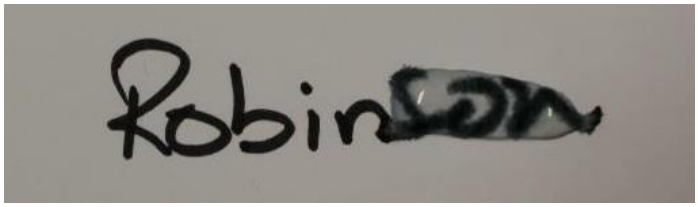


Figure 77 - Procedure point 2

3. At 2cm from the pointed end of the strip, put the chromatography paper on top of the wet letters to absorb the ink from the paper, and gently press the chromatography paper with your fingers [Figure 78].

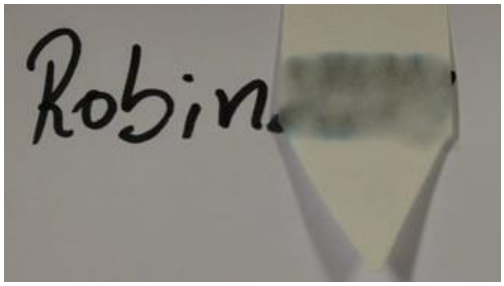


Figure 78 - Procedure point 3

4. For the Evidence#7 and Evidence#10, write the same as Evidence#6 on a piece of paper. The piece of paper should be similar to the suspect document.
5. Repeat steps 2 to 3.
6. Label your strips at the top with a pencil so you can tell them apart.
7. In the beaker, put 30ml of ethanol and 10ml of water
8. Put the strips inside the beaker and tape them with adhesive tape to the top of the beaker so they do not move. The strip should be in contact with the solution, while keeping the dot above the solvent level and without touching the walls of the beaker.
9. Cover the beaker with a watch glass or some other object
10. Wait 35 minutes to allow the solvent to soak up the strip.

11. After that time, remove the strips from the beaker and place a mark where the solvent reached. Then allow the filter paper to dry.
12. Record your work in Table 13.

Table 13 - Table of test results – example data

Substance	Evidence#6			Evidence#7			Evidence#10		
	Distance travelled by substance (cm)	Distance travelled by solvent (cm)	R_f Value	Distance travelled by substance (cm)	Distance travelled by solvent (cm)	R_f Value	Distance travelled by substance (cm)	Distance travelled by solvent (cm)	R_f Value
Substance 1	*4.6*	*5.5*	*0.84*	*3.2*	*5.4*	*0.59*	*4.6*	*5.5*	*0.84*
Substance 2	*5.5*	*5.5*	*1*	*4.3*	*5.4*	*0.80*	*5.5*	*5.5*	*1*
Substance 3	----	----	----	*5.4*	*5.4*	*1*	----	----	----

Notes for Teachers

The values of Table 12 and Table 13 are examples of possible results.

Using Table 13, students should compare the R_f value in order to determine which pen was used to write the victim's name (the pen used was Evidence#10).

Example of the chromatograms obtained [Figure 79].

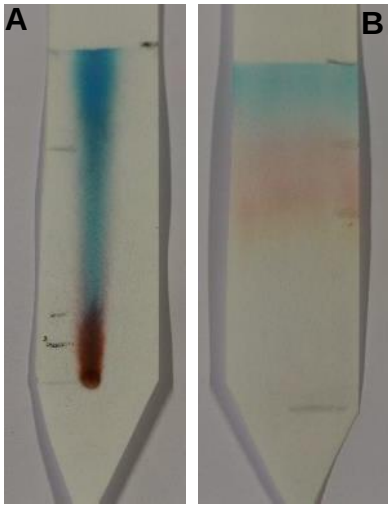


Figure 79 - (A) chromatogram using the pen; (B) chromatogram using the document

Questions and Answers:

1. Indicate the three types of chromatography.

Thin layer chromatography (TLC), liquid chromatography and gas chromatography.

2. Explain the basic principle of TLC (paper chromatography).

Paper chromatography is a form of chromatography where the substances of a mixture get separated. The substances will have different affinities for the mobile and stationary phases, and this affects the speed at which it travels.

3. Explain why it is necessary to cover the developing chamber during the paper development.

This step is essential as the environment inside the chamber should remain saturated with the solvent vapour.

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Links to UK GCSE Science Curriculum

Forensic Activity: Human Individual Identification	Apparatus & Techniques	Working Scientifically	Related to: Core Practicals/ RPs/ PAGs	Scientific concepts
Activity I: Is this Really Blood?	AT3 - Chemistry AT6 - Chemistry AT8 – Biology	KS3 – 2.4, 2.5, 3.3 KS4 – 2.4, 2.6, 3.5	AQA – RP7 Chy Edexcel – 9.6C OCR – PAG C5	Oxidation – reduction reactions and catalysts
Activity II: Blood Typing Analysis	AT6 - Chemistry AT8 - Biology	KS3 – 2.4, 2.5, 3.3 KS4 – 2.4, 2.6, 3.5	AQA – RP7 Chy Edexcel – 9.6C OCR – PAG B2 & C5	Antigens and antibodies used in the process of blood typing
Activity III: DNA Profiling	AT4 - Chemistry	KS3 – 2.4, 2.5, 3.3 KS4 – 1.4, 2.4, 2.6, 3.5, 3.6	AQA – RP12 Edexcel – 2.11 OCR – PAG C3	Genetics – DNA structure
Activity IV: Comparison of different fingerprint patterns	AT7 - Biology	KS3 – 1.1, 2.5, 3.4 KS4 – 1.4, 2.5, 2.6, 3.4, 3.6, 3.7	OCR – PAG B1	Unique nature of fingerprints and how to interpret them
Activity V: Dusting and lifting Latent Fingerprints	AT7 - Biology	KS3 – 2.4, 3.3 KS4 – 2.4, 3.5	N/A	Unique nature of fingerprints and how to lift them
Activity VI: Revealing Latent Fingerprints using Iodine Fuming	AT6 - Chemistry	KS3 – 2.4 KS4 – 2.4	N/A	Unique nature of fingerprints and how to visualise latent prints

Forensic Activity: Environment Education	**Apparatus & Techniques**	**Working Scientifically**	**Related to Core Practicals/ RPs/ PAGs**	**Scientific concepts**
Activity VII: The Hidden Secrets of Pollen Grains	AT7 - Biology	KS3 – 2.4, 2.6 KS4 – 2.4, 2.6, 3.5, 3.6	AQA – RP1 Biology Edexcel – 1.6 OCR – PAG B1	Making observations Reproduction in plants Habitats and ecology
Activity VIII: The Insect Clock	AT3 - Biology	KS3 – 2.2, 3.1 KS4 – 2.2, 3.1, 3.5, 3.6	N/A	Life cycles Decay
Activity IX: Hair and Footprints	AT7 – Biology	KS3 – 2.6, 3.5 KS4 – 2.6, 3.5, 3.6	AQA – RP1 Biology Edexcel – 1.6 OCR – PAG B1	Specialised cells Habitats
Forensic Activity: Health Education	**Apparatus & Techniques**	**Working Scientifically**	**Related to Core Practicals/ RPs/ PAGs**	**Scientific concepts**
Activity X: Alcohol effect on liver	AT3 – Biology AT5 – Biology AT6 - Chemistry	KS3 – 2.4, 2.6, 3.1, 3.3, 3.4 KS4 – 2.4, 2.6, 3.1, 3.3, 3.4, 3.5, 3.6	AQA – RP3 Biology Edexcel – 1.16 OCR – PAG B3	Osmosis Non communicable diseases Lifestyle and health
Activity XI: Sugar in Blood	AT2 – Biology/ Chemistry	KS3 – 2.4, 3.5 KS4 - 2.4, 3.5, 3.6	AQA RP4 Biology Edexcel – 1.13B OCR – PAG B8	Using reagents to tests for carbohydrates Lifestyle and health
Activity XII: Invisible Ink	AT6 – Chemistry	KS3 – 2.4 KS4 – 2.4	N/A	Oxidation Electromagnetic spectrum
Activity XIII: The colour of Guilt – Chromatography	AT4 – Chemistry	KS3 – 2.4, 2.6, 3.1, 3.3, 3.4 KS4 – 1.4, 2.4, 2.6, 3.3, 3.5, 3.6	AQA – RP12 CS – Chy Edexcel – 2.11 OCR – PAG C3	Paper chromatography

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Working Scientifically

The tables below show examples of the ways working scientifically could be assessed.
The column to the right gives examples of what students could be asked to do in an exam.

1 Development of scientific thinking

WS 1.1	Understand how scientific methods and theories develop over time.	Give examples to show how scientific methods and theories have changed over time. Explain, with an example, why new data from experiments or observations led to changes in models or theories. Decide whether or not given data supports a particular theory.
WS 1.2	Use a variety of models such as representational, spatial, descriptive, computational and mathematical to solve problems, make predictions and to develop scientific explanations and understanding of familiar and unfamiliar facts.	Recognise/draw/interpret diagrams. Translate from data to a representation with a model. Use models in explanations, or match features of a model to the data from experiments or observations that the model describes or explains. Make predictions or calculate quantities based on the model or show its limitations. Give examples of ways in which a model can be tested by observation or experiment.
WS 1.3	Appreciate the power and limitations of science and consider any ethical issues which may arise.	Explain why data is needed to answer scientific questions, and why it may be uncertain, incomplete or not available. Outline a simple ethical argument about the rights and wrongs of a new technology.
WS 1.4	Explain every day and technological applications of science; evaluate associated personal, social, economic and environmental implications; and make decisions based on the evaluation of evidence and arguments.	Describe and explain specified examples of the technological applications of science. Describe and evaluate, with the help of data, methods that can be used to tackle problems caused by human impacts on the environment.
WS 1.5	Evaluate risks both in practical science and the wider societal context, including perception of risk in relation to data and consequences.	Give examples to show that there are hazards associated with science-based technologies which have to be considered alongside the benefits. Suggest reasons why the perception of risk is often very different from the measured risk (eg voluntary vs imposed risks, familiar vs unfamiliar risks, visible vs invisible hazards).
WS 1.6	Recognise the importance of peer review of results and of communicating results to a range of audiences.	Explain that the process of peer review helps to detect false claims and to establish a consensus about which claims should be regarded as valid. Explain that reports of scientific developments in the popular media are not subject to peer review and may be oversimplified, inaccurate or biased.

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2 Experimental skills and strategies

WS 2.1	Use scientific theories and explanations to develop hypotheses.	Suggest a hypothesis to explain given observations or data.
WS 2.2	Plan experiments or devise procedures to make observations, produce or characterise a substance, test hypotheses, check data or explore phenomena.	Describe a practical procedure for a specified purpose. Explain why a given practical procedure is well designed for its specified purpose. Explain the need to manipulate and control variables. Identify in a given context: • the independent variable as the one that is changed or selected by the investigator • the dependent variable that is measured for each change in the independent variable • control variables and be able to explain why they are kept the same. Apply understanding of apparatus and techniques to suggest a procedure for a specified purpose.
WS 2.3	Apply a knowledge of a range of techniques, instruments, apparatus, and materials to select those appropriate to the experiment.	Describe/suggest/select the technique, instrument, apparatus or material that should be used for a particular purpose, and explain why.
WS 2.4	Carry out experiments appropriately having due regard for the correct manipulation of apparatus, the accuracy of measurements and health and safety considerations.	Identify the main hazards in specified practical contexts. Suggest methods of reducing the risk of harm in practical contexts.
WS 2.5	Recognise when to apply a knowledge of sampling techniques to ensure any samples collected are representative.	Suggest and describe an appropriate sampling technique in a given context.
WS 2.6	Make and record observations and measurements using a range of apparatus and methods.	Read measurements off a scale in a practical context and record appropriately.
WS 2.7	Evaluate methods and suggest possible improvements and further investigations.	Assess whether sufficient, precise measurements have been taken in an experiment. Evaluate methods with a view to determining whether or not they are valid.

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3 Analysis and evaluation

WS 3.1	Presenting observations and other data using appropriate methods.	Construct and interpret frequency tables and diagrams, bar charts and histograms. Plot two variables from experimental or other data.
WS 3.2	Translating data from one form to another.	Translate data between graphical and numeric form.
WS 3.3	Carrying out and represent mathematical and statistical analysis.	For example: • use an appropriate number of significant figures • find the arithmetic mean and range of a set of data • construct and interpret frequency tables and diagrams, bar charts and histograms • make order of magnitude calculations • change the subject of an equation • substitute numerical values into algebraic equations using appropriate units for physical quantities • determine the slope and intercept of a linear graph • draw and use the slope of a tangent to a curve as a measure of rate of change • understand the physical significance of area between a curve and the x-axis and measure it by counting squares as appropriate.
WS 3.4	Representing distributions of results and make estimations of uncertainty.	Apply the idea that whenever a measurement is made, there is always some uncertainty about the result obtained. Use the range of a set of measurements about the mean as a measure of uncertainty.
WS 3.5	Interpreting observations and other data (presented in verbal, diagrammatic, graphical, symbolic or numerical form), including identifying patterns and trends, making inferences and drawing conclusions.	Use data to make predictions. Recognise or describe patterns and trends in data presented in a variety of tabular, graphical and other forms. Draw conclusions from given observations.
WS 3.6	Presenting reasoned explanations including relating data to hypotheses.	Comment on the extent to which data is consistent with a given hypothesis. Identify which of two or more hypotheses provides a better explanation of data in a given context.

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WS 3.7	Being objective, evaluating data in terms of accuracy, precision, repeatability and reproducibility and identifying potential sources of random and systematic error.	Apply the following ideas to evaluate data to suggest improvements to procedures and techniques. • An accurate measurement is one that is close to the true value. • Measurements are precise if they cluster closely. • Measurements are repeatable when repetition, under the same conditions by the same investigator, gives similar results. • Measurements are reproducible if similar results are obtained by different investigators with different equipment. • Measurements are affected by random error due to results varying in unpredictable ways; these errors can be reduced by making more measurements and reporting a mean value. • Systematic error is due to measurement results differing from the true value by a consistent amount each time. • Any anomalous values should be examined to try to identify the cause and, if a product of a poor measurement, ignored.
WS 3.8	Communicating the scientific rationale for investigations, methods used, findings and reasoned conclusions through paper-based and electronic reports and presentations using verbal, diagrammatic, graphical, numerical and symbolic forms.	Present coherent and logically structured responses, using the ideas in 2 Experimental skills and strategies and 3 Analysis and evaluation, applied to the required practicals, and other practical investigations given appropriate information.

4 Scientific vocabulary, quantities, units, symbols and nomenclature

WS 4.1	Use scientific vocabulary, terminology and definitions.	The knowledge and skills in this section apply across the specification, including the required practicals.
WS 4.2	Recognise the importance of scientific quantities and understand how they are determined.	
WS 4.3	Use SI units (eg kg, g, mg; km, m, mm; kJ, J) and IUPAC chemical nomenclature unless inappropriate.	
WS 4.4	Use prefixes and powers of ten for orders of magnitude (eg tera, giga, mega, kilo, centi, milli, micro and nano).	
WS 4.5	Interconvert units.	
WS 4.6	Use an appropriate number of significant figures in calculation.	

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Appendix 4

List of apparatus and techniques

The following list includes opportunities for choice and use of appropriate laboratory apparatus for a variety of experimental problem-solving and/or enquiry based activities.

Safety is an overriding requirement for all practical work. Centres are responsible for ensuring appropriate safety procedures are followed whenever their students complete practical work.

Use and production of appropriate scientific diagrams to set up and record apparatus and procedures used in practical work is common to all science subjects and should be included wherever appropriate.

	BIOLOGY	CHEMISTRY	PHYSICS
1	Use of appropriate apparatus to make and record a range of measurements accurately, including length, area, mass, time, temperature, volume of liquids and gases, and pH	Use of appropriate apparatus to make and record a range of measurements accurately, including mass, time, temperature, and volume of liquids and gases	Use of appropriate apparatus to make and record a range of measurements accurately, including length, area, mass, time, volume and temperature. Use of such measurements to determine densities of solid and liquid objects.
2	Safe use of appropriate heating devices and techniques including use of a Bunsen burner and a water bath or electric heater	Safe use of appropriate heating devices and techniques including use of a Bunsen burner and a water bath or electric heater	Use of appropriate apparatus to measure and observe the effects of forces including the extension of springs
3	Use of appropriate apparatus and techniques for the observation and measurement of biological changes and/or processes	Use of appropriate apparatus and techniques for conducting and monitoring chemical reactions, including appropriate reagents and/or techniques for the measurement of pH in different situations	Use of appropriate apparatus and techniques for measuring motion, including determination of speed and rate of change of speed (acceleration/deceleration)
4	Safe and ethical use of living organisms (plants or animals) to measure physiological functions and responses to the environment	Safe use of a range of equipment to purify and/or separate chemical mixtures including evaporation, filtration, crystallisation, chromatography and distillation	Making observations of waves in fluids and solids to identify the suitability of apparatus to measure speed/frequency/wavelength. Making observations of the effects of the interaction of electromagnetic waves with matter.
5	Measurement of rates of reaction by a variety of methods including production of gas, uptake of water and colour change of indicator	Making and recording of appropriate observations during chemical reactions including changes in temperature and the measurement of rates of reaction by a variety of methods such as production of gas and colour change	Safe use of appropriate apparatus in a range of contexts to measure energy changes/transfers and associated values such as work done
6	Application of appropriate sampling techniques to investigate the distribution and abundance of organisms in an ecosystem via direct use in the field	Safe use and careful handling of gases, liquids and solids, including careful mixing of reagents under controlled conditions, using appropriate apparatus to explore chemical changes and/or products	Use of appropriate apparatus to measure current, potential difference (voltage) and resistance, and to explore the characteristics of a variety of circuit elements
7	Use of appropriate apparatus, techniques and magnification, including microscopes, to make observations of biological specimens and produce labelled scientific drawings	Use of appropriate apparatus and techniques to draw, set up and use electrochemical cells for separation and production of elements and compounds	Use of circuit diagrams to construct and check series and parallel circuits including a variety of common circuit elements

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Spin Off of a Forensic Science Educational Strategy Promoting Scientific Literacy and European Students Citizenship

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FORENSIC TOOLBOX

Coordinator:

universidade de aveiro dbio departamento de biologia

Partners:

inova skipton girls' high school INnCREASE EEO GROUP PLAY SOLUTIONS