Lid
(Support for paper)
Chromatography Paper or TLC plate
Original sample
Solvent
Solvent front
Separated components

2014-2015
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Staff

The following staff are associated with this course:

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(Module Leader)

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Demonstrators: Emma Garrett.

Safety and Laboratory Guidelines

It is essential to adopt a positive approach to safety. No list of safety rules can cover all eventualities. The design of each experiment, even of a simple test tube reaction, must include a consideration of the hazards involved. In addition to the departmental instructions on safety your attention is drawn particularly to the following points:

- You have a legal obligation to work safely, to ensure that neither you nor your co-workers are exposed to hazards, and to comply with the safety regulations issued to you at the beginning of the year.
- You must look up all Risk and Safety statements for each chemical you use to comply with the Control of Substances Hazardous to Health (COSHH) regulations.

Hazard and precaution statements can be found at:

The H and P statements are also in the Lennard-Jones Laboratories safety handbook.

- Protective and suitable clothing must be worn at all times when working in the laboratory, this includes:
  - Lab coat
  - Safety spectacles
- Nitrile gloves when handling corrosive material
- Shoes that do not expose any bear skin and heels only 2” or below.
- Clothing that does not expose any bear legs, NO shorts or skirts.
- All long hair must be tied back
- NO contact lenses

➢ When working in the lab you must not:
  - Eat or drink, this includes chewing gum
  - Use mobile phones or MP3 players

➢ Please ensure you know the location of your closest fire exit.

**IF IN DOUBT ABOUT ANY SAFETY OR LABORATORY RULES PLEASE ASK A MEMBER OF STAFF.**

**Attendance**

Attendance at practical sessions is **compulsory**.

You must arrive in good time for the commencement of a laboratory session.

It is required that your completed laboratory notebook is signed by a member of staff at the end of each laboratory session.
Assessment Timetable

Assessment of the practical component is 30% of your overall module mark.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Type of Assessment</th>
<th>Weighting (%)</th>
<th>Deadline</th>
</tr>
</thead>
<tbody>
<tr>
<td>LABORATORY 2 – CHROMATOGRAPHY</td>
<td>Pre lab Pro-forma</td>
<td>20%</td>
<td>Group A: Mon 23/02/15</td>
</tr>
<tr>
<td></td>
<td>Lab report</td>
<td></td>
<td>Group B: Mon 26/02/15</td>
</tr>
<tr>
<td>LABORATORY 3 – DNA ANALYSIS</td>
<td>Lab report</td>
<td>10%</td>
<td>Group A: Mon 20/04/15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Group B: Mon 23/04/15</td>
</tr>
</tbody>
</table>

In addition to the summative assessment of the practical work there will also be formative assessment of laboratory 1 (Blood analysis) and your laboratory notebook.
Pre-Laboratory Preparation Guidelines

One of the most important aspects of being a scientist is maintaining a laboratory notebook;

➢ Before coming to a laboratory session your laboratory Notebook you should have the following:
   ▪ Date
   ▪ Title of the Experiment
   ▪ Experimental Aim and specific objectives.
   ▪ Make preliminary notes (calculations, reaction schemes, design of the apparatus, brief procedure) which may help you with the experimental work.
   ▪ Safety section detailing any chemicals used.
   ▪ Address any pre-laboratory work.

Laboratory Notebook Guidelines

One of the most important aspects of being a scientist is recording observations. These should be written directly into your laboratory notebook while work is being carried out in the laboratory. For legal reasons at the end of the day work should be signed and dated and countersigned by a member of staff. This will also give them a chance to comment upon your note keeping and to suggest improvements. Results and data collected are then written up as reports or pro-formas as required.

Organisation and Guidelines for a laboratory Notebook

➢ Full name and contact address on front page.
➢ Contents page with list of experiments and pages numbers.
➢ All pages should be numbered, NEVER tear pages from your laboratory notebook.
➢ Your notes should be precise, clear and legible.
➢ Someone should be able to open your lab notebook on any page and find out what you did on a particular day.
- **NO** pencil, all work should be written in pen.
- **NO** “tippex” or other corrective marker should be used, any mistakes should be crossed using a single line.

For each experiment your notebook should include:
- Title and date.
- Aims and objectives.
- Additional notes such as a reaction scheme or design of the apparatus.
- Safety section, including necessary COSHH forms.
- Procedure, particularly anything that differs from the lab script.
- Observations.
- Results, including any pictures taken of you agarose gel.
- Calculations.
- Conclusion and any literature references.
- Your signature/date and countersigned signature of a member of laboratory staff.
# Laboratory Schedule

Lab sessions will be in the Lennard-Jones Laboratories. Lab sessions will be on either a Monday (Group A) or Thursday (Group B) afternoon, see below for specific times.

<table>
<thead>
<tr>
<th>Week</th>
<th>Monday (Group A)</th>
<th>Thursday (Group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No lab session</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No lab session</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Blood analysis pre-lab (VC) Main Library PC lab 3 <strong>14.30-17.00</strong></td>
<td>Blood analysis Pre-lab (VC) Main Library PC lab 3 14.00-16.30</td>
</tr>
<tr>
<td>4</td>
<td>Blood analysis practical (VC) LJ1.70 <strong>14.30-17.00</strong></td>
<td>Blood typing practical (VC) LJ1.70 14.00-16.30</td>
</tr>
<tr>
<td>5</td>
<td>Thin layer chromatography practical (DFT) LJ1.80 14.00-17.00</td>
<td>Thin layer chromatography practical (DFT) LJ1.60 14.00-17.00</td>
</tr>
<tr>
<td>6</td>
<td>Electrophoresis practical (DFT) LJ1.80 14.00-17.00</td>
<td>Electrophoresis practical (DFT) LJ1.60 14.00-17.00</td>
</tr>
<tr>
<td>7</td>
<td>No lab session</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>DNA analysis pre-lab (AVC/VC) LJ1.70 14.00-17.00</td>
<td>DNA analysis pre-lab (AVC/VC) LJ1.70 14.00-17.00</td>
</tr>
<tr>
<td>9</td>
<td>DNA analysis practical (AVC/VC) LJ1.70 14.00-17.00</td>
<td>DNA analysis practical (AVC/VC) LJ1.70 14.00-17.00</td>
</tr>
<tr>
<td><strong>Easter</strong></td>
<td>No lab sessions</td>
<td>No lab sessions</td>
</tr>
<tr>
<td>Week</td>
<td>Monday</td>
<td>Thursday (Group A &amp; B)</td>
</tr>
<tr>
<td>10</td>
<td>No lab session</td>
<td></td>
</tr>
</tbody>
</table>
| 11   | No lab session | Forensic Anthropology lecture (VC) 15.00-16.00  
Revision session (VC) 16.00-17.00  
CBA 1.103 |
| 12   | Bank Holiday - No lab session | Forensic Anthropology Workshop (DR)  
Group A 14.00-15.30  
Group B 15.30-17.00  
LJ1.70 |
LABORATORY 1 - BLOOD ANALYSIS

GUIDELINES FOR WEEK 3 PRE-LABORATORY SESSION

In week 3 you will be having a pre-lab session.

Group A Monday 9th Feb Main Library PC Lab 3 (2.30-5 pm)
Group B Thursday 12th Feb Main Library PC Lab 3 (2-4.30 pm)

At the end of this session you will be able to:

1. Use search engines to look for a specific journal article and accurately use search terms when researching a new topic.
2. Describe the theory behind presumptive blood tests used at crime scenes and the molecular theory of ABO blood typing (Week 4’s experiment will involve determining the ABO blood group from an unknown blood sample).
3. Demonstrate that you can cite and reference all sources used to answer pre-lab questions and apply this knowledge to future coursework.
4. Utilise marking criteria guides that are applied to your work.
5. Locate a hard copy paper from the archive of the Keele library.

Session Preparation

The session will be 2.5 hours long and at the end of the session you will need to hand in your answered proforma for evaluation and feedback. The questions you need to answer can be found in a document on the KLE > Content > Laboratory information > Week 3 library pre-lab session > Pre-lab session week 3 pro-forma 14_15). This document also includes a reference to a journal which should help you when answering question 1.

To enable you to complete all 3 questions and your references by the end of the session in week 3 you will need to attempt all questions before coming to the pre-lab session in week 3. You may also find it useful to bring along hard copies of the sources you have used to
answer your questions. I would advise you to complete the pro-forma in an electronic format so that it can be edited and added to during the session and printer credit to print off your work, there will be computers available for you to do this.

NOTE: FORENSIC IDENTIFICATION USING PROTEINS, WITH EMPHASIS ON USE OF THE ABO BLOOD TYPING METHOD IN FORENSICS WILL BE ASSESSED SUMMATIVELY IN THE END OF MODULE EXAMINATION (WORTH 50% OF THE MODULE MARKS).
LABORATORY 1 - BLOOD ANALYSIS

ABO BLOOD GROUP TYPING PRE-LABORATORY
PREFERENCES

1) Complete Table 1 below for the luminol test for presumptive blood identification. Explain how the luminol test works on a molecular level, and the advantages and disadvantages of this test. (20 marks)

Expand table as required.

<table>
<thead>
<tr>
<th>EXPLANATION</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 marks</td>
<td>5 marks</td>
<td>5 marks</td>
</tr>
</tbody>
</table>

2) How would you ascertain that blood at the crime scene is of human origin? (15 marks)

Expand table as required.
3) Explain the principles behind ABO blood typing and how the test works on a molecular level. (Include genetic inheritance of the blood groups in your answer) (20 marks)

Expand table as required.

REFERENCES (10 marks)

Include a list of all sources used. For an example of how to cite and reference your work see journal provided:

Total marks (65)
LABORATORY 1 - BLOOD ANALYSIS

BLOOD ANALYSIS PRACTICAL

Introduction

The presence of blood in a crime scene is useful as it would link suspect and victim. Hence the importance of blood testing and blood stain analysis. Blood takes a few days to degrade and needs to be tested as soon as it is found in a crime scene. Blood can be tested by serological, biochemical, and/or DNA typing methods. Blood itself consists of water, cells (red and white cells, platelets), enzymes, protein factors, glucose, and ions. Turbid yellow plasma consists mainly of water and clear yellow serum, which in turn, contains water, antibodies, growth factors, hormones, white cells, and platelets. DNA typing is performed on white cells that contain nuclear DNA.

Presumptive tests for blood

Red blood cells are the most abundant cells in blood and contain haemoglobin that transports oxygen to and removes toxic carbon dioxide from tissues. Most presumptive tests for blood are a result of a chemical reaction with this iron-containing protein. The appearance of blood can be easily recognised, red if it’s fresh whereas older stains may appear a reddish-brown. However other liquids such as paint, rusty water, food colouring, and sauces may also be mistaken for blood. Performing a presumptive blood test can indicate if the stain is actually blood.

Most of the presumptive blood tests used in forensic science rely on oxidation-reduction reactions. Oxidation-reduction reactions can be defined as one molecule loosing electrons (oxidised) and another molecule gaining electrons (reduced).

Heme the major component of haemoglobin has an activity in presumptive tests of a class of enzymes known as peroxidases. A peroxidase can reduce hydrogen peroxide (H₂O₂) to water (H₂O) while oxidising the electron donor. This reaction is used in many presumptive blood tests such as Hemastix®.
Luminol reagent is a mixture of three different chemicals, luminol powder (yellowish crystal), hydrogen peroxide ($\text{H}_2\text{O}_2$) and hydroxide salt. Luminol starts as a reduced molecule but under the catalyst heme loses hydrogen and nitrogen atoms and gains oxygen to become a new energised and oxidised compound (3-aminophthalate). When this energy is released the result is the observation of a blue/green visible light.

Some other stains can induce a similar result (false positives), such as plants (horseradish) and potatoes that contain similar enzymes. Luminol also reacts with copper, urine, fecal matter and some bleaches.

**Blood group typing**

Red blood cells display many proteins, (more than 256), on their surface. Each one of these proteins can be recognised with the appropriate antibody. Therefore, these proteins are antigenic or red blood cell antigens. Blood typing may have been surpassed by DNA typing but it is very valuable in some cases. Identical twins will have identical DNA profile but might display different antibodies in their serum. In the ABO system for blood typing, (1901), blood groups are defined by the type of antigen(s) on their red blood cell (see table below) and their specific reaction with cognate anti-sera. Anti-serum against A antigen ($\alpha_A$) or anti-serum against B antigen ($\alpha_B$) reacts specifically with A or B antigen respectively (see Table 1). These anti-sera are commercially available.

**Table 1: Red blood cell agglutination in ABO blood typing**

<table>
<thead>
<tr>
<th>Group</th>
<th>Antigen A</th>
<th>Antigen B</th>
<th>Agglutination by sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>-</td>
<td>$\alpha_A$</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td>$\alpha_B$</td>
</tr>
<tr>
<td>AB</td>
<td>+</td>
<td>+</td>
<td>$\alpha_A, \alpha_B$</td>
</tr>
<tr>
<td>O</td>
<td>-</td>
<td>-</td>
<td>Neither</td>
</tr>
</tbody>
</table>
An additional red blood cell marker is the D antigen or *Rhesus* factor (a protein also found in *Rhesus* monkeys). The Rh system classifies blood types into positive (~85%) and negative. Blood types are approximately distributed in the population, (although there are important geographical and ethnic variations), as shown in the Table 2.

Further subtypes are obtained using lectins, proteins (from plant seeds, commercially available) that recognise specific sugars in these antigens since the proteins contain sugars (glycoproteins). For example, it has been estimated that 80% of the A type blood population is A1 while the remaining 20% is A2.

<table>
<thead>
<tr>
<th>Table 2. Percentage of each ABO blood type in selected countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO Blood group</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>O</td>
</tr>
<tr>
<td>AB</td>
</tr>
</tbody>
</table>

NB: Data accurate to the nearest percentage. ABO group and sub-group percentage distribution vary between ethnic groups.

Source: Adapted from Give blood website at [http://www.blood.co.uk/about-blood/blood-group-basics/](http://www.blood.co.uk/about-blood/blood-group-basics/)[accessed 05/01/2015](http://www.blood.co.uk/about-blood/blood-group-basics/)(last updated 03/2014)

**Aims & Expected Learning Outcomes**

This practical aims to demonstrate the usefulness of the luminol as a presumptive test for blood and also the ABO system for human blood group typing. At the end of the practical you should be able to:

- Discuss the use of luminol at a crime scene.
- Explain how the luminol test is performed.
• Describe the immunological basis of the ABO group typing for human blood used in forensic serology.
• Apply the ABO method to serologically type human blood cell samples.

NOTE: FORENSIC IDENTIFICATION USING PROTEINS, WITH EMPHASIS ON USE OF THE ABO BLOOD TYPING METHOD IN FORENSICS WILL BE ASSESSED SUMMATIVELY IN THE END OF MODULE EXAMINATION (WORTH 50% OF THE MODULE MARKS).

Safety
Blood potentially contains pathogenic microorganisms or viruses (including hepatitis B and human immunodeficiency virus).

Blood for this practical is from the Midlands and South West National Blood Service, and has tested negative for HIV 1 and HIV2, Hepatitis B and C and TP. **This does not mean that screened blood is negative for all known blood-borne pathogens.**

Refer to COSHH/risk assessment form for the further information on luminol.

Biological Assessment
The legislation that applies to work with human blood is The control of Substances Hazardous to Health Regulations 2002. Under these regulations, we have to ensure safe working with human blood by:

a) carrying out a risk assessment before starting work;
b) adopting appropriate precautions to eliminate or minimise the risk to health;
c) implementing adequate supervision to ensure precautions are used;
d) maintaining, inspecting and testing the effectiveness of precautions;
e) ensuring staff/students work safely by providing them with information, instruction and training.
General Measures

Handling of screened blood samples requires safety containment class 2. This means that:

a) Staff/students must receive training in working safely with human blood.

b) A high standard of supervision of the work should be maintained.

c) Accidents/incidents should be reported immediately to, and recorded by, the Principal Tutor and the Health & Safety Officer.

Physical Measures

a) Access to the Lennard Jones laboratory during the session is restricted to laboratory Tutors and the CHE-10042 group only.

b) A biohazard sign is to be displayed on each bench.

c) There should be adequate space for each worker.

d) The working area is protected by absorbent paper backed with plastic impervious to water.

e) Before exiting the lab, workers must wash hands in a wash basin located near the rear of the laboratory.

f) An autoclave for the sterilisation of waste materials is available in the same building.

Operating Procedures

a) The laboratory door is closed when work is in progress.

b) Laboratory coats must be worn and removed when leaving the laboratory. Your coat will be inspected by a Tutor for any evidence of contamination. If contamination is present, you will need to leave the lab coat in the laboratory for disinfection, autoclaving and washing.

c) Eating, chewing, drinking, smoking, taking medication, storing food and cosmetic application must not take place in the laboratory.

d) Mouth-pipetting must not take place.

e) Hands must be washed or disinfected when contamination is suspected, after handling blood and before leaving the laboratory.

f) You will be using vinyl gloves and laboratory specs during the laboratory session. Do change gloves before handling personal items or items likely to be touched by others.
g) In general, work can be carried out on the open bench but care must be taken to minimise the production of aerosols. The bench working area is delimited within the benchkote absorbent area. **Do not place lab books, scripts etc on this working area.** Likewise, do not work outside the delineated working area.

h) Bench surfaces should be regularly decontaminated taking into account the pattern of work.

i) Effective disinfectants are available for routine disinfection, and for immediate use in the event of a spill.

j) There are means for the safe collection, storage and disposal of contaminated waste:
   a) Disposable gloves, contaminated absorbent blue paper & disposable aprons: autoclave waste bags.
   b) Disposable Pasteur pipettes, contaminated wooden cocktail sticks and used slides soak in disinfectant, making sure pipettes are immersed.
   c) Universals and Eppendorf tubes: leave on the bench for disposal by trained staff.

**Procedure for spills**

Inform immediately the nearest Tutor who will assess the nature of the incident/accident. Since you will be handling micro-volumes of blood, the procedure for decontaminating small spills will be followed:

   a) spray with appropriate disinfectant (one spray bottle per bench).
   b) mop up with blue paper towels.
   c) discard contaminated absorbent paper and contaminated gloves into biohazard autoclave waste bags.
   d) spray bench with disinfectant solution and leave for 10min before drying with paper towels.
   e) Discard paper towels in biohazard autoclave waste bags.
Practical Outline

Case

Tim C, a security guard in a Bank, was shot dead from a bullet wound on 30th January 2005. Police found a bullet lodged in one of the ceiling tiles. Forensic specialists examined the bullet and found it contained blood. Two days later, police discovered an abandoned car in a river nearby. The police traced the car to its owner who was questioned at his home. The man appeared to be ill and in pain. Police noticed a bloody coat folded on a chair, but the man did not answer any questions about it. The police took the bloody coat away for forensic examination as well as other items of clothing.

Part A: Blood group analysis

You are a forensic investigator and have found that Tim C’s blood group type is O⁺.

You are now going to type the blood found in the bullet and on the bloody coat of the suspect to try to answer whether or not the suspect is involved in this crime.

Apparatus and Materials

- ABO typing kit (A, B AB and O type blood samples and anti-sera against A and against B blood cells, NHS, Central Pathology Laboratory)
- Microscopic slides
- Plastic disposable Pasteur pipettes
- Wax markers
- wooden toothpicks
- Benchkote protective paper/tape
- Virkon disinfectant in beakers and sprayer bottles
- Appropriate protective clothing (lab coats) disposable lab aprons, safety glasses and nitrile gloves.
- Permanent marker pens.
The Tutor will briefly remind you of the theory behind ABO blood group typing already presented during Lecture 3 in week 2 and explain how the running of the session.

You will need to **design and prepare the experimental plan** for this practical in your Laboratory Notebook, including the construction of a Table, appropriate for recording the results to be obtained.

You might find the following experimental requirements useful for preparing the design and table:

- You need to carry out **reference control reactions** with anti-sera against red blood cells antigen A (αA, blue colour antisera solution) and with anti-sera against B antigen (αB, yellow coloured anti-sera solution) to check agglutination reagents with known blood group samples (four blood type samples A, B, AB and O, each to be tested against each of two anti-sera (αA and αB).

- Once you have observed that your controls have given expected positive **AND** negative reactions and you feel confident that the anti-sera you have used distinguishes between positive and negative agglutination patterns, you need to carry out the tests of the two forensic trace blood samples: Bu# (Bullet) & S#(Suspect), each to be tested against each of two anti-sera (αA and αB).

Organise your bench working area according to your experimental plan.

**DO NOT START THE AGGLUTINATION REACTIONS BEFORE BOTH, HAVING IT CHECKED WITH YOUR TUTOR AND OBSERVING A DEMONSTRATION OF THE PROCEDURE.**

Test ABO type reference blood, Bullet and Suspect samples, using the demonstrated procedure. Check your results or any doubts about the procedure with your Tutor.
Record your results and group number (e.g. Bu13 etc) in your Laboratory notebook.
Ensure that you have your Table of results and conclusion checked by your Tutor.

Part B: Luminol Demonstration
After examination of the blood found on the coat recovered from the suspects house and the blood found on the bullet another white T-shirt is submitted for analysis. There is a very small reddish-brown stain that needs examining.

Presumptive blood test demonstration
Your tutor will show you how to perform a presumptive blood test on this item using the luminol test.

In your lab book record each step of the analysis and the results. Ensure that you have your results and conclusion checked by your Tutor.

Recommended Reference books and related links


Chasteen, T.G. Luminol and TCPO Chemiluminescence factsheet.
Available at www.shsu.edu/~chm_tgc/JPPdir/JPP1999/
(Last updated 1999) [Accessed 08/01/15]


LABORATORY 2 - CHROMATOGRAPHY

WEEK 5 PRE-LABORATORY WORK

These questions need to be answered and handed in BEFORE you enter the lab.

Chromatography Pre-Lab QUESTIONS

1. Give the reaction scheme for the reaction of ninhydrin with amino acids.
   (5 marks)

2. The forensic lab has analysed a sample on the presence/absence of cocaine with TLC. A drawing of the TLC plate is given below.
   
   ![TLC Plate Diagram]

   a) Calculate the R_f factor for cocaine.
   b) Can you confirm that the unknown sample contains cocaine? Explain why or why not.
   c) Give two main disadvantages of TLC.
   (10 marks)

These questions need to be answered and handed in BEFORE you enter the lab.
Part A: Thin Layer Chromatography

Introduction

Chromatographic separation methods exploit the relative affinities of the components of a mixture for two phases termed the stationary phase (paper, silica gel, alumina etc.) and the mobile phase (a liquid solvent (or solvent mixture) or a gas). The mobile phase, containing the solutes to be separated, passes over (or through) the stationary phase. The various components of the mixture (carried by the mobile phase) progress through the stationary phase, but at different rates according to how strongly each component interacts with the stationary phase. For each solute the process of passing through the stationary phase is equivalent to a succession of solvent extractions. Various chromatographic techniques exist and you are encouraged to read up on these using the references given below or any other source of material you can find on this topic.

Thin layer chromatography (TLC) is an easy, convenient and inexpensive way to determine how many components are in a mixture and, in many instances, can be used to identify the components as well. In today’s experiments, you will gain experience with thin layer chromatography, you will work with a variety of developing solvents, and you will use several different techniques to visualize the spots of a chromatogram.

Paper chromatography, which can also be used to separate amino acids, is actually a form of partition chromatography, rather than adsorption chromatography. Water, a component of the developing solvent, forms hydrogen bonds with the fibres of the paper and serves as the stationary phase. The organic liquids that are also present in the developing solvent serve as the mobile phase. The components of the mixture are drawn up the paper to different heights, depending on their solubility in the mobile phase. The compounds that are more soluble in
the organic liquid remain dissolved in the mobile phase longer than those that are less soluble and thus travel further up the paper.

Amino acids are colourless compounds. In order to see the spots on the chromatogram, you will apply a solution of ninhydrin to the TLC plate. Ninhydrin will react with the amino acid to produce a purple compound.

Figure 1: Apparatus for paper chromatography or TLC.
**Aims**
In the first part of this experiment the technique of **thin layer chromatography** (TLC) is used to separate and identify a series of amino acids. The aim for this experiment is to identify the amino acids present in an unknown sample. In TLC an aluminium slide coated with a thin layer of silica gel (the stationary phase) is used.

**Safety**
Refer to COSHH/risk assessment form for the further information on this experiment.

*Caution: This experiment uses harmful and flammable solvents - perform the work in a fume cupboard, avoid open flames and wear gloves.*

**Practical Outline (Work in pairs):**

**Part A: Thin Layer Chromatography Practical Outline (work in pairs)**

**Apparatus and Materials**
- tall beaker
- watch glass
- 5 x 10 cm thin-layer plate
- mobile phase (solvent mixture (butanol:acetic acid; water (4:1:1))
- glass capillary tubes
- 0.1% ninhydrin solution.

a) **Wearing gloves**, obtain a 5 x 10 cm TLC plate. With a pencil, lightly draw a line parallel to the short edge of the TLC plate (approximately 1 cm from the short edge). Mark the positions for the five spots on each plate, spaced equally, where the samples will be spotted. **Be sure not to touch the TLC plates without gloves because amino acids from the hand will contaminate the plate.**

b) Using separate, clean spot applicators spot a little of each amino acid solution onto the 5 x 10 cm TLC plate. In addition, place a spot of your unknown solution on the TLC plate at the same distance from the plate edge.
c) Run the chromatogram in a tall beaker containing the mobile phase using a watch glass for a lid. Ensure that the solvent surface lies below the line of sample spots (a solvent depth of 0.5 cm should be adequate).

d) The solvent front must not advance up to or beyond the edge of the plates. When the solvent front nears the edge of the plates, about 1-2 cm from the edges, remove the plate from the beaker. **Immediately** mark with a pencil the position of the solvent front.

e) When the chromatogram is completely dry visualize the spots with the 0.1% ninhydrin solution (**make sure you are wearing gloves**). Place the TLC plate on a watch glass to dry. When the TLC plate is dry, circle the spots with a pencil.

f) Measure the distance (in cm) travelled by the solvent front from the line of original sample spots.

**Treatment of results – Part A**

Calculate the $R_f$ values of all the components present on your chromatogram (show your working out).

Identify the amino acid(s) in your unknown sample. Include a sketch of your TLC plate in your laboratory report. You should also include any observations you have made during the session.

**Part B: Solid Phase Extraction**

**Introduction**

Solid phase extraction (SPE) is a powerful method for sample preparation and is used by most chromatographers today. It has capabilities in a broad range of applications such as environmental analyses, pharmaceutical and biochemical analyses, organic chemistry and food analyses.

The advantages of SPE compared to classical liquid-liquid extraction are the low solvent consumption, the enormous time saving and the potential for automation. Additionally, a
sample preparation task can often be solved more specifically by using SPE, since different interactions of the analyte with the solid stationary phase (adsorbent) are possible, and methods can be optimised by adjusting chromatographic conditions. SPE offers a multitude of adsorbents for polar, hydrophobic and/or ionic interactions, while liquid-liquid extraction is limited to partition equilibriums in the liquid phase. SPE provide rapid, economical and effective systems for sample preparation. They can be used to process samples for HPLC, GC, TLC, UV or IR spectroscopy and many more. For the increasingly sensitive chromatographic analyses good sample preparation is essential, because it protects the chromatographic columns, and it allows a greater sensitivity by removal of interfering matrix components. A selective and specific sample preparation thus is a prerequisite for reasonable, economical and sensitive analyses.

The main objectives of SPE are removal of interfering matrix components and selective concentration and isolation of the analytes. Enrichment can increase the detection sensitivity by a factor of 100 to 5000. Often this step is necessary to reach the concentration limit of detection for the analytes of interest for qualitative or quantitative analyses, i.e. without enrichment often a reliable analysis at trace level is not possible.

In general, SPE can be used for three important purposes in up-to-date analyses:

- concentration of the analyte
- removal of interfering substances
- changing the matrix of the analyte as needed for subsequent analyses

In most cases these three effects occur together. Since analytes can be either adsorbed on the SPE packing material or directly flow through, while the interfering substances are retained, two general separation procedures are possible. The first case is shown in the figure below.
The sample is pressed or drawn through the solid phase, and the analyte molecules are enriched on the adsorbent. Interfering components and solvent molecules (matrix) are not retained. Then remaining interfering components are washed from the adsorbent with a suitable washing solution. Finally, the analyte is removed from the adsorbent by elution with a suitable solvent.

In some cases other interfering components may remain on the adsorbent. Such a strong adsorption of interfering components offers another possibility for the pre-purification of difficult matrices, such as waste oils or sludge. If the analytes show no interaction with the adsorbent and if only the interfering components are retained, the solid phase can be used to simply "filter" the sample, as shown in the figure below.
In SPE there are three main types of interactions between the analyte and the stationary phase (adsorbent). These are nonpolar, polar and ionic interactions.

Non-polar
Nonpolar interactions occur between hydrocarbon residues of the functional groups of the adsorbent and the analyte. Since most organic compounds have a nonpolar structure, they can adsorbed to nonpolar adsorbents via van-der-Waals forces.
Almost all organic compounds have a certain potential for nonpolar interactions. Exceptions are compounds possessing a large number of polar or even ionic groups, which shield the nonpolar character of the carbon skeleton (e.g. carbohydrates). Typical adsorbents with a pronounced nonpolar character are e.g. C18ec, C18, C18 Hydra and C8 modified silicas. They show a seemingly low selectivity, because their functional groups, the alkyl substituents, can interact with almost all nonpolar analytes. This can be used for the isolation of substance groups of different structure.

Polar
Polar interactions include hydrogen bonds, dipole-dipole and $\pi-\pi$ interactions, which can occur between many different adsorbents and functional groups of the analytes. Some of these interactions are possible between amino, hydroxyl and carbonyl groups as well as aromatic rings, double bonds and groups with hetero-atoms such as nitrogen, sulphur, phosphorus and oxygen. Typical adsorbents for polar interactions are unmodified silica, CN, NH$_2$ and OH (diol) modified silicas.

In general, polar compounds are easily adsorbed to a polar adsorbent from a nonpolar environment and are eluted with a polar solvent. The opposite holds true for nonpolar compounds. They are easily adsorbed from a polar environment onto nonpolar surfaces. Elution is achieved by solvents of lower polarity.
Ionic

Ionic interactions occur between charged analytes and an adsorbent with a functional group of opposite charge. Cationic groups are present in primary, secondary, tertiary and quaternary amines and inorganic cations, e.g. calcium, sodium, magnesium etc. Examples for anionic groups are carboxylic and sulphonic acids, phosphates and similar groups.

Retention via these ion exchange interactions is enhanced in a matrix of low ionic strength and a counter ion of low selectivity (e.g. acetate, Na\(^+\)). For elution a solvent with high ionic strength and high selectivity is preferred (e.g. citrate or Ca\(^{2+}\)).

*Adjusted with permission from: “Solid Phase Extraction Application Guide”, Macherey-Nagel*

Aims

The aim of this part B is to use SPE and TLC to determine if a serum sample contains caffeine and/or paracetamol.

Part B: SPE Practical Outline

Apparatus and Materials

- tall beaker
- watch glass
- 5 x 6 cm thin-layer plate
- mobile phase (solvent mixture 80% of ethyl acetate and 20% of petroleum ether)
- glass capillary tubes
- C18 SPE cartridge
- syringe adapter
- 5 mL syringe
- 10 mL volumetric flask (x2)
- 2 mL serum/tris buffer sample
- 3 mL methanol
- 2 mL tris buffer
- deionised water
- caffeine and paracetamol.
Solid Phase Extraction (SPE)

1. Sample Pre-treatment: Mix 1 mL serum with 1mL 0.1M Tris Buffer, pH 7 (*This step has already been done for you*)

2. Column Conditioning: Condition each column with methanol (2 × 1 mL)^a

3. Column Equilibration: Equilibrate each column with 0.1M Tris Buffer, pH 7 (2 × 1 mL)^a,b

4. Sample Application: Load your sample on the column and slowly force the solution through^a

5. Interference Elution: Elute interferences with deionised water (1 mL)^a

6. Drying: Dry the cartridge by pushing air through it (5 x 5 mL) using your empty syringe.

7. Analyte Elution: Elute analytes with methanol (1 mL). Allow elution solvent to soak into sorbent for 0.5-1 min **before** eluting the analytes^a.

8. Analysis: Analyse your sample for the presence of caffeine or paracetamol with TLC

^a Use flow rates up to 1-2mL/min. See also Figure 2.

^b A thin film of solvent should remain on the stationary phase after you add the aqueous conditioning solvent. This promotes better contact between an aqueous sample matrix and the hydrophobic solid phase
Thin Layer Chromatography (TLC):
You will need to prepare a solution of caffeine and paracetamol (separate solutions) containing approximately 40 mg of either caffeine or paracetamol in 10 mL of ethyl acetate. These will be spotted on to your TLC plate below.

Together with your sample from the SPE also spot the following solutions on the TLC plate:
1) Caffeine solution
2) Paracetamol solution
3) Solution of your own paracetamol (ca. 2mg in 0.5mL ethyl acetate, produced in CHE 10038)

You should end up with four spots on the TLC plate (you may wish to double check this by viewing your TLC plate under UV light before continuing).

This part of the experiment must be carried out in a fume cupboard.

a) Run the TLC plate in a beaker containing 5-10 ml of the following solvent system:
   80% of ethyl acetate and 20% of petroleum ether. Cover the beaker with a watch glass.
b) Examine the TLC plate under UV light. Compounds that absorb UV radiation appear as dark spots against a light background. Draw a line around the spots with a pencil.
c) Calculate the retention factor, $R_f$, value for each component of the mixture.

**Treatment of Results – Part B**

1) Determine whether your serum sample contained caffeine and/or paracetamol
2) Visually determine the purity of your own synthetic paracetamol.

These results will need to be written up as a lab report which will be due in on Monday/Thursday (depending on your group) of week 8 before 3 pm. Guidelines for this report are detailed below along with the weight of marks attached to each section.

**Recommended Reading**

*Quantitative Chemical Analysis*: Daniel Harris, (2010)
LABORATORY 2 - CHROMATOGRAPHY

CHROMATOGRAPHY LAB REPORT GUIDELINES

Your clear and concise lab report should contain the following sections.

- **Introduction (10 %)** to your report should outline the principles and theory behind the experiment, and state explicitly the aims of the experiment. This should be original and not just a copy of the information provided in the laboratory script.

- **Experimental Plan (15 %)** should be a brief account of what you did and observed while carrying out the experiment. It should only contain a (bullet point) plan and research strategy of your work. Do not copy the procedure from the lab script.

- **Results and Discussion (50 %)** section should contain all your results (spectra, tables, graphs, pictures etc.) and data analysis, showing the units for all quantities. These should be presented in a clear and logical manner, so that it is possible to follow your work. Discussion should be limited to answers to all aims and (if required) additional questions followed by a very brief critical assessment of your data and the experimental method.

- **Conclusion (15 %)** should summarise what the experiment has shown and reflect on whether the aims of your work have been achieved.

- **References (10 %)** section should list published materials which you have used in your work with the correct formatting.

Word count for this lab report is ca. 1500 words

You will also be marked on the presentation of the lab report. Is it clearly written as a report (not as a script), are all figures necessary and properly labeled, have you used tables and graphs properly (if needed), are references included where needed, etc.?
Introduction

Electrophoresis is a separation technique based on the movement of charged ions under the influence of an electrical field. This technique is commonly used for the separation of amino acids and peptides on the basis of their charge. All amino acids contain ionizable groups that cause the amino acids, in solution, to act as charged polyelectrolytes that can migrate in an electric field. The amino acids with a net positive charge will migrate toward the negative electrode. Those with a negative net charge will move toward the positive electrode. An inert substance such as paper or gel is used as a supporting medium for the conducting liquid in most electrophoretic methods. In this experiment of separating amino acids, a phosphate/borate buffer will be used as the conducting liquid and cellulose as the supporting medium.

Aims & Expected Learning Outcomes

The purpose of this experiment is to determine the effect of an electrical field on charged particles and to use this information to identify the amino acids present in an unknown sample.

Safety and Practical Outline

A detailed procedure will be provided before the laboratory session along with a COSHH/risk assessment form.

Recommended Reading

*Quantitative Chemical Analysis*; Daniel Harris, (2010)
LABORATORY 3 – DNA ANALYSIS
WEEK 8 PRE-LABORATORY SESSION

In this pre-lab session we will be going over the background for the lab class in week 9, including calculation of the reaction components and doing some preparatory genetics questions. This is to enable you to start the lab work as soon as you get into the lab so that we can have the gels running as soon as possible.

Mendelian and Population Genetics Questions.

Attempt questions 1-3 first. You will then be given some further background information to help you with questions 4-6.

To be able to understand the inheritance patterns of the genetic markers used in forensic analysis, and how they can be applied to produce discriminatory profiles, it is essential that you have a firm grasp of Mendelian and population genetics. The following questions will test your knowledge of the rules of inheritance.

1) A couple have 2 children, one of whom has the recessive condition cystic fibrosis (CF). They would like to have another child and have visited a genetic counsellor to find out the likelihood of having another affected child.

   a) What is the chance that they will have an affected child, what is the chance that the child will be a carrier and what is the chance that it will be normal (ie two dominant alleles) ?

   b) What is the likelihood that their unaffected child is a carrier?

2) 10 unrelated black curly haired mice are crossed with 10 unrelated white short haired mice. All the offspring have black curly hair. When the offspring are then crossed together (taking care not to cross pups from the same litter), a total of 120 pups are
produced. 79 are black and curly, 23 are white with short hair, 8 are black with short hair and 10 are white with curly hair.

a) Are these the results you expect?

b) Can you explain any discrepancy?

3) A woman has 5 children, 2 daughters and a son from her first marriage and another son and daughter from her second marriage. All of her children appear to have inherited her serious visual defect. Her five children all have 1 child. Her daughters’ children also have the defect, but her sons’ children have perfect vision.

How do you explain this observation?

4) 

Calculate the fraction of genes the following individuals have in common
1 and 2; 1 and 3; 1 and 4; 2 and 4; 3 and 4

5) Blood type is determined by the three alleles for the protein agglutinogen. The alleles for the genes encoding agglutinogens A, B and O are present at the ratio of approximately 25:5:70, respectively, in the UK population. A and B are codominant whilst O is recessive.
If your suspect has blood type A how many people would you expect to have the same blood type in a population of 2500?

6) For genetic marker A, the allele frequencies are:

A1) 0.5 A2) 0.25 A3) 0.1 A4) 0.15

For Genetic marker B, the allele frequencies are

B1) 0.8 B2) 0.1 B3) 0.1


In a population of 1000, how many people would be expected to have the same genotype for A?

In a population of 1000, how many people would be expected to have the same genotype for B?

In a population of 1000, how many people would be expected to have the same genotype for A and B combined?
**LABORATORY 3 – DNA ANALYSIS**

**WEEK 9 DNA ANALYSIS PRACTICAL**

**Introduction**

DNA fingerprinting has been used for a number of years to help identify suspects by matching DNA samples found at crime scenes with DNA from the suspect.

VNTR based DNA fingerprinting was developed before the advent of PCR (that forms the basis of most current fingerprinting protocols) and requires µg quantities of DNA, which may not be available from some forensic samples. The DNA is digested with restriction enzymes, run on an agarose gel, Southern blotted and hybridised with probes that recognise minisatellite loci to detect minisatellite RFLPs. Restriction enzymes cut the DNA at specific recognition sites. Minisatellites are repeated sequences found at various locations in the genome and thus one probe may hybridise too many bands on a Southern blot, each band representing a restriction fragment carrying a copy of that particular minisatellite sequence. Minisatellites are extremely variable, so no two individuals are likely to have the same sets of bands when several different minisatellite probes are used.

With the advent of PCR, DNA fingerprinting became possible on even very minute samples of DNA. PCR based fingerprinting is based on the amplification of multiple microsatellite (STR) loci using sets of primers. For each primer pair, a maximum of 2 bands will be amplified per individual, depending on whether they are homozygous or heterozygous at each locus. These microsatellite loci are hyper variable in the population so no two individuals are likely to produce the same sized bands by PCR for all the loci tested. Most DNA profiles are produced in this way now, using kits with about 13 different sets of marker loci.

Microsatellites and minisatellites are inherited in Mendelian fashion and thus close relatives will share some, but not all bands in common.
This practical is a simulation of DNA fingerprinting, using restriction enzyme digestion of DNA – you’ll be doing PCR based fingerprinting next year. The enzyme you will be using today is a second generation restriction enzyme that has been engineered to fully digest samples in about 10 minutes, as opposed to several hours. In a real life situation a restriction enzyme digest of genomic DNA would produce a smear of bands on an agarose gel and you would have to do a southern blot and use minisatellite probes to detect the bands. In this instance however the DNA sample you will be provided with will only produce a few distinct bands on the gel.

**Aims & Expected Learning Outcomes**

The aim of this practical is to analyse several DNA samples by restriction enzyme digestion to identify the prime suspect in a crime. This will involve setting up digests, running a gel and interpreting the gel image.

You will practise a range of technical and arithmetical skills, including handling small volumes using micropipettes, and handling agarose gels.

**Safety**

This experiment has been assessed for the purposes of the COSHH regulations. All the procedures have been assigned at a risk level of low, with the exception of those involving the following reagents:

<table>
<thead>
<tr>
<th>Ethidium Bromide</th>
<th>⚠️ Danger ⚠️</th>
</tr>
</thead>
<tbody>
<tr>
<td>H: 302, 330, 341</td>
<td>P: 260, 281, 284, 310</td>
</tr>
</tbody>
</table>

1. Wear safety glasses and gloves when handling solutions containing this reagent.
2. All waste solutions containing ethidium bromide must be disposed of in the waste containers provided.
3. Ensure long hair is properly tied back when loading gels.
Practical Outline

Case
A young woman has been murdered. Clearly she put up a fight as she has skin cells from her attacker under her nails. 3 suspects, each with a large scratch somewhere on their body (face, forearm or back of hand) have been arrested. It is your job to nominate the prime suspect. You have been provided with DNA samples extracted from the victim, the 3 suspects and from the cells found under the victim’s nails. You will digest each DNA sample with a restriction endonuclease and you will separate the fragments on an agarose gel to reveal the pattern of bands produced in each case. By comparing the band patterns, you should be able to pick out your prime suspect.

Apparatus and Materials

- DNA samples from the victim and the suspects (2 µL at 0.25 µg/µL)
- 100 µL sterile distilled water
- 15 µL FastDigest Green buffer (10X concentration) – the buffer provides the necessary conditions for the enzyme to work and also contains a density reagent and dyes so that the sample can be directly loaded onto the gel, post-digestion.
- 7 µL FastDigest enzyme (PstI) at 1 unit/µL (1 unit is defined as the amount required to digest 1µg of DNA in 5 minutes).
- Pre-cast gel (0.8% agarose in TBE buffer)
- TBE running buffer (89mM Tris, 89mM borate, 2mM EDTA, pH8.0)
- 15 µL size marker (1kb GeneRuler ladder)

Restriction-digestion set-up
You need to set up 5 separate reactions, each in a final volume of 20 µL. The tubes already contain the DNA.

Reaction 1 contains 2µL DNA sample (victim), 1 unit of enzyme, FastDigest Green buffer at a final concentration of 1X and water to a final volume of 20µL.
Reaction 2 contains 2µL DNA sample (suspect 1), 1 unit of enzyme, FastDigest Green buffer at a final concentration of 1X and water to a final volume of 20µL.

Reaction 3 contains 2µL DNA sample (suspect 2), 1 unit of enzyme, FastDigest Green buffer at a final concentration of 1X and water to a final volume of 20µL.

Reaction 4 contains 2µL DNA sample (suspect 3), 1 unit of enzyme, FastDigest Green buffer at a final concentration of 1X and water to a final volume of 20µL.

Reaction 5 contains 2µL DNA sample (sample from under victim’s nail), 1 unit of enzyme, FastDigest Green buffer at a final concentration of 1X and water to a final volume of 20µL.

You will have calculated the volumes of each component you need to add in the pre-lab session in week 8.

Please ensure that you label your tubes well with your initials, as your samples will be placed in the incubator you will need to be able to identify them.

You will be using micropipettes with a maximum volume of 10 µL, so any components that are in larger volumes will need to be added in two aliquots. When working with such small volumes it is vitally important that you use the micropipettes properly. Set the pipette to the correct volume. Attach a fresh sterile tip. Expel the air by pushing the piston to the first position and keep the piston depressed until you have placed the tip below the surface of the liquid. (In this case we are expelling the air before entering the tip to avoid creating air bubbles in the small volumes of stock solution.) Slowly release the piston to suck up the liquid. Expel the liquid into the tube by pushing the piston down as far as it will go. (If some of the liquid appears to be stuck inside the tip, gently remove the tip and put it back on again, then depress the piston once more.) If in doubt – ask a demonstrator.

a) **Keeping the reaction tubes on ice at all times**, prepare your 5 reactions. To minimize the difference in time between the addition of the enzymes to each tube, add the appropriate volumes of water, then buffer and finally the enzymes to the 5 reaction tubes. It is very important to avoid cross contamination of the samples or the stock solutions so take a fresh tip every time.
b) Mix the tubes by vortexing and spin briefly in a microfuge to collect the sample at the bottom of the tube.

c) Place the tubes in the 37°C incubator for 10 minutes

d) After 10 minutes, remove your tubes from the incubator.

Running the gel

You will now run your enzyme digested samples on an agarose gel to separate out the different size fragments. When using standard restriction enzymes it is usual to add a viscous coloured loading dye to the samples – however the FastDigest Green buffer used when you set up your digests not only ensures the correct reaction conditions for the enzyme to work, but also contains a viscosity agent and a marker dye to help the DNA sink into the wells and allow you to track the progress of the sample through the gel.

For safety and time reasons, the gels have been pre-cast and contain ethidium bromide (EtBr). Gels were prepared by melting the agarose powder (0.8% w/v) in gel running buffer and allowing it to cool so to hand heat. At this point EtBr to a final concentration of 0.2 µg/mL was added and the gel was poured into the casting tray and the comb inserted. An alternative to adding the EtBr to the gel is to submerge the gel in a 0.5 µg/ml solution of EtBr after electrophoresis - this gives a much cleaner photograph of the gel, but is a more hazardous procedure.

a) Submerge the gel in gel running buffer – the gel should be no more than a few mm below the surface of the buffer.

b) Carefully remove the comb.

c) Using a micropipette, load the samples into the wells (20 µL), taking a note of which sample is loaded into which well.

d) Alongside your samples load the size marker - load 15µL into the well.

Place the lid on the tank and attach the electrodes to the power supply (see figure 1 below). Set to 100V max for 1 – 1.5 hours. You can check the progress of the electrophoresis by seeing how far the colour has migrated.
Figure 1 – Schematic of a gel electrophoresis system. Taken from J.M. Butler, Fundamentals of Forensic DNA Typing, Academic Press, London and California, 2010, p177.

e) While the gel is running attempt exercises 1, 2 and 3 from DNA Analysis proforma.

f) Photograph the gel under UV light.

g) In your lab book record each step of the analysis and the results. Ensure that you have your results and conclusion checked by your Tutor.

The DNA analysis proforma should be completed by typing your answers directly on to the proforma. To attach your gel picture, you may need to scan it first or alternatively you can draw the picture of your gel.

You need to submit it online, via the link on the KLE page. You will receive audiovisual feedback.

Recommended Reading


LABORATORY 3 – DNA ANALYSIS

DNA ANALYSIS PROFORMA

Name

Student number:

Please complete the following proforma and submit it for marking via the assignment submission link on the KLE page. A sample of the scripts will be marked using audiovisual feedback. This counts for 10% of the module mark.

Experimental

COMPLETE THE FOLLOWING TABLE (10 marks)

<table>
<thead>
<tr>
<th>component</th>
<th>Stock solution Conc</th>
<th>Volume added to tubes</th>
<th>Concentration (eg amount per unit volume) or amount (mg, no of units etc) in final reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA* (See below)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FastBuffer Green</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme (Pst I)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>N/A</td>
<td></td>
<td>N/A</td>
</tr>
</tbody>
</table>

*State which DNAs were in reactions 1-5 (2.5 marks).

1
2
3
4
5
1) Why do you add FastDigest Green buffer to the DNA sample? (3 marks)

2) Why does DNA migrate towards the +’ve electrode? (2 marks)

3) Why do smaller DNA fragments move more quickly through a gel than larger ones? (2 marks)

4) 0.8% agarose is ideal for separating fragments in the size range 0.5-8kbp. If you wanted to separate fragments in the range 0.1 to 1kbp would you use a higher or lower agarose concentration and why? (1.5 marks)

5) What type of gel can be used to give good resolution of small fragments that may differ in size by only a few bases? (1 mark)
Results
Insert a fully labeled copy of your gel photo here (or draw a copy of it- no need to include the marker track if you draw it). (4 marks)

Interpretation of results
Can you identify a prime suspect? Explain your reasoning (2 marks)

The sample taken from under the victim’s nails has more bands than any of the suspects. How can you explain these extra bands? (2 marks)
Exercise 1

The following diagram shows minisatellite DNA fingerprinting

(a) The panel shows the fingerprints from a mother (M) and her child (C) and 2 possible fathers (F1 and F2). Which man is the father? Justify your answer. (3 marks)

(b) The panel shows DNA fingerprints of a victim, 3 suspects and a blood sample (the victim punched her attacker, causing his nose to bleed) found at the crime scene. Which, if any of the 3 suspects were at the crime scene? (2 marks)
Exercise 2

7 women have been raped and from the descriptions given by the victims, police suspect that all 7 crimes were perpetrated by the same man. Semen was recovered from swabs of all 7 victims. The police now have 3 suspects in custody and have taken DNA samples. Using PCR, 6 separate microsatellite loci were amplified. The panels below, show the bands amplified by each set of primers for the 3 suspects and from the semen samples from the victims.

1) How does the evidence suggest that the police right in suspecting that the same person committed all the crimes? (2 marks)

2) Suggest why the bands from victims 1 and 2 less intense than those for the other victims. (2 marks)
3) Who is the prime suspect – justify your answer? (2 marks)

4) Can you be absolutely certain that this is the perpetrator of the crimes? (3 marks)

5) Why do you think that more than one microsatellite locus has been studied? (3 marks)

6) Why does each probe produce 2 bands? (3 marks)

7) Can you think of any situations where you might only see 1 band – hint think of the chromosomal differences between males and females? (6 marks)
Exercise 3

For genetic marker A, the allele frequencies are:
A1) 0.4 A2) 0.25 A3) 0.2 A4) 0.15

For Genetic marker B, the allele frequencies are:
B1) 0.7 B2) 0.1 B3) 0.2

For genetic marker C, the allele frequencies are:
C1) 0.5 C2) 0.25 C3) 0.01 C4) 0.24


In a population of 1000, how many people would be expected to have the same genotype for A?

In a population of 1000, how many people would be expected to have the same genotype for B?

In a population of 1000, how many people would be expected to have the same genotype for C?

In a population of 1000, how many people would be expected to have the same genotype for A and B and C combined?

(10 marks)
On Thursday of week 12 there will be a Forensic Anthropology workshop delivered by a guest lecturer. The workshop will be 1.5 hrs long and you will be spilt into two groups.

Group A Thursday May 7th LJ1.70 14.00-15.30
Group B Thursday May 7th LJ1.70 15.30-17.00

More details regarding this session will be provided closer to the date.

**Recommended Reference books and related links**


