Edexcel International AS Biology

Your notes

Plants & Bacterial Growth

Contents

Your notes

How Bacterial Growth Can Affect Plants

- Bacteria are microscopic **prokaryotes** (meaning their cells do not contain a nucleus or membrane-bound organelles)
- Some may **cause disease** in both plants and animals
- Bacteria are often used in studies to test the antimicrobial properties of substances
  - **Antimicrobial** means that the substance will either **kill microbes** (i.e. bacteria) or **prevent their growth**
- In order to perform these investigations, it is important to create an environment with all the **conditions** necessary for **bacterial growth** to occur
- Bacteria require the following conditions to survive and reproduce:
  - They require **nutrients**, which provide them with the materials needed to **grow** and **respire**
  - Those that respire **aerobically** will need a sufficient supply of **oxygen**
  - The **temperature** and **pH** of the environment must not be too high or too low to allow **enzymes** that control metabolic processes to **function optimally**

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Plant Products with Antimicrobial Properties

- Plants are **susceptible to attack** by micro-organisms, such as bacteria and fungi
- This can cause **damage** to the plant and may even **kill** them
- Plants have developed a range of **chemical defences** against these microbes
  - These may include **antiseptic compounds** and **antibiotics**
- Antimicrobial chemicals from plants can be exploited by humans to **develop new drugs** to treat bacterial and fungal diseases
- It is possible to produce a **plant extract** containing these antimicrobial chemicals to test its effect on the growth of bacteria

Other therapeutic drugs sourced from plants

- Plants may also produce a variety of **other compounds** that can **treat a range of ailments** in humans, from pain killers to cancer drugs
  - Scientists were able to **extract the active ingredient** from willow bark, used by people for pain relief over the centuries, which led to the development of modern-day **aspirin**
  - **Quinine** is a drug sourced from the cinchona tree, used to treat and prevent malaria
- An **advantage** of extracting and purifying active plant compounds is the ability to produce medication that contains a **known concentration** of the active ingredient
  - This ensures a **reliable dosage** of medication
- **Analysing** the chemical structure of the active ingredients enables scientists to create **synthetic versions** of the compound in a laboratory
  - This **reduces** the need to **remove large amounts** of plant material from the environment
  - **Modifications** can be made in the laboratory to make the active ingredient **more effective**
- The development of therapeutic drugs from plant products has enabled humans to be more successful at treating diseases

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Antimicrobial Properties of Plants

- Certain plant species have the ability to **kill** or **prevent the growth** of micro-organisms
- These **antimicrobial properties** can be incorporated into the development of new drugs

Apparatus

- Broth containing bacterial culture and nutrients
- Agar plate
- Pipette
- Plastic spreader
- Plant tissue
- Pestle and mortar
- Ethanol
- Funnel
- Glass beaker
- Filter paper
- Forceps
- Stopwatch
- Incubator

Method

1. Prior to this practical, bacteria would have been grown in a mixture of distilled water and nutrients, along with a specific bacterial culture
    - This mixture is called a **broth**
2. **Transfer** some of the bacteria from the broth onto an agar plate (which is a petri dish filled with agar jelly that will serve as a growth medium for the bacteria) using a **sterile pipette**
3. Make sure the bacteria is **evenly spread out** by using a **sterile plastic spreader**
    - Open the lid of the of the agar plate **as little as possible** when doing this to avoid contaminating the plate with other fungi or bacteria present in the surrounding air
    - Place the lid back on top of the agar plate **immediately afterward** to prevent contamination
4. To prepare the plant extracts, plant tissue must be dried and ground finely

5. This should be **soaked in ethanol** to extract the antimicrobial substances, after which it should be filtered
6. Equal sized discs cut from **sterile absorbent paper** should be dipped in the plant extract using sterile forceps
7. Leave the discs in the extract for the **same amount of time** to ensure that they absorb a similar amount of the plant extract
    - The disc that will serve as the **control** will only be dipped in ethanol
8. Space the discs out evenly on the agar plate, before taping the lid on, inverting the plate and **incubating it at 25°C**
    - This temperature will ensure good bacterial growth without stimulating the growth of human pathogens
9. Incubate for 24 to 48 hours

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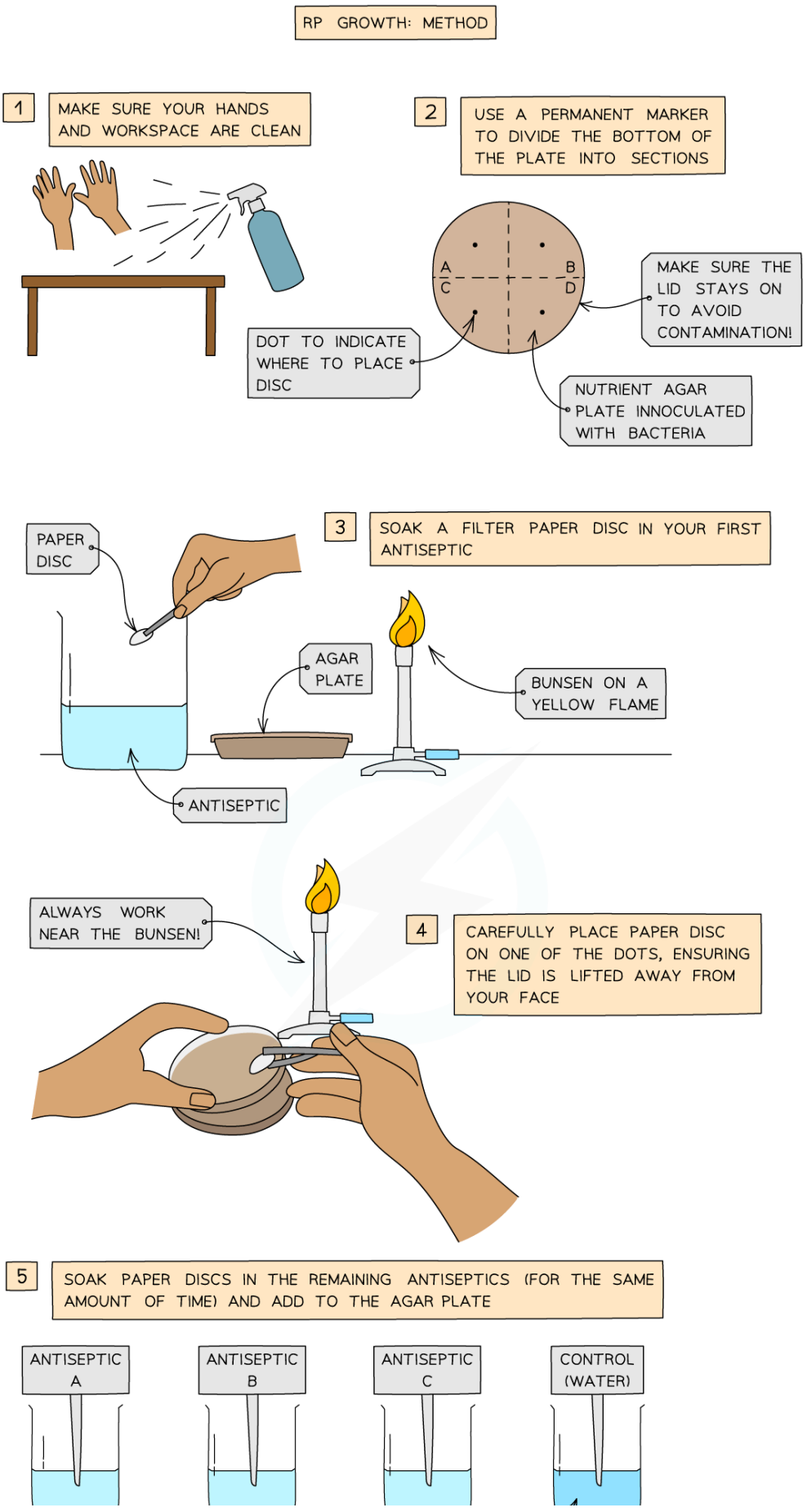
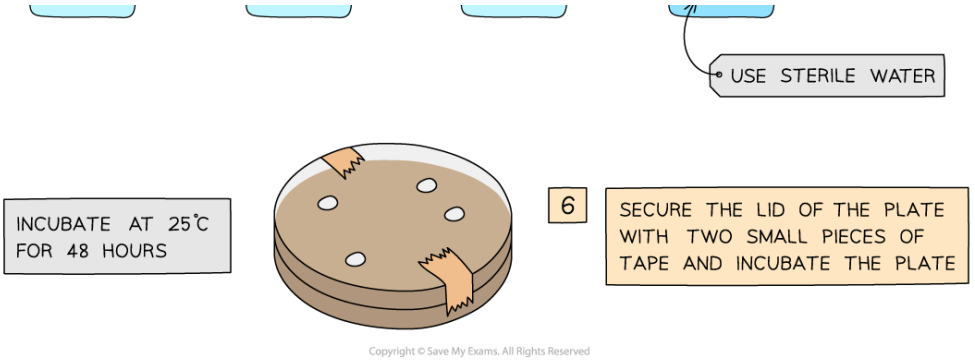

RP GROWTH: METHOD
1
MAKE SURE YOUR HANDS AND WORKSPACE ARE CLEAN
2
USE A PERMANENT MARKER TO DIVIDE THE BOTTOM OF THE PLATE INTO SECTIONS
A
B
C
D
MAKE SURE THE LID STAYS ON TO AVOID CONTAMINATION!
DOT TO INDICATE WHERE TO PLACE DISC
NUTRIENT AGAR PLATE INNOCULATED WITH BACTERIA
PAPER DISC
3
SOAK A FILTER PAPER DISC IN YOUR FIRST ANTISEPTIC
AGAR PLATE
BUNSEN ON A YELLOW FLAME
ANTISEPTIC
ALWAYS WORK NEAR THE BUNSEN!
4
CAREFULLY PLACE PAPER DISC ON ONE OF THE DOTS, ENSURING THE LID IS LIFTED AWAY FROM YOUR FACE
5
SOAK PAPER DISCS IN THE REMAINING ANTISEPTICS (FOR THE SAME AMOUNT OF TIME) AND ADD TO THE AGAR PLATE
ANTISEPTIC A
ANTISEPTIC B
ANTISEPTIC C
CONTROL (WATER)



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The same method as that shown above can be used to investigate the antimicrobial properties of plants. Just remember that the paper discs are soaked in different plant extracts (instead of different antiseptics) and the control disc should be soaked in ethanol (instead of sterile water)

Analysis

- The area around each disc where bacteria cannot grow is known as the **clear zone**
- The **larger** the clear zone, the **more effective** the antimicrobial properties of that plant extract was
- The size of the clear zone can be determined by **measuring the diameter** or by **calculating the area** (area = πr^2)
- **Repeat** the experiment at least three times and **calculate the mean** of the results

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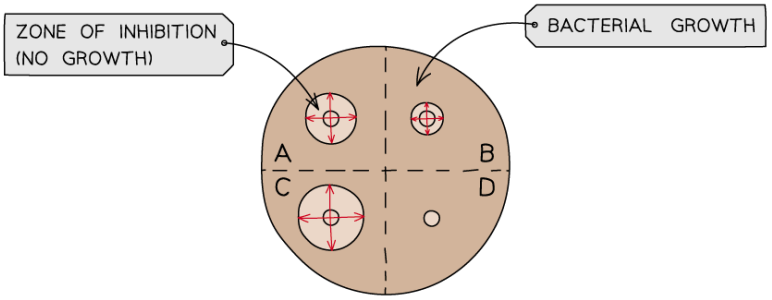
RP GROWTH: ANALYSIS

1 MEASURE THE DIAMETER OF THE CLEAR ZONE AROUND EACH DISC. REPEAT MEASUREMENT AT A 90° ANGLE



2 CALCULATE THE MEAN DIAMETER OF EACH ZONE AND FROM THESE VALUES CALCULATE THE CROSS-SECTIONAL AREA OF THE CLEAR ZONES AROUND THE BACTERIAL COLONIES

TYPE OF ANTISEPTIC	DIAMETER OF CLEAR ZONE (mm)			MEAN RADIUS (mm)	CROSS SECTIONAL AREA (mm^2)
	1	2	MEAN		
A	17.0	15.0	16.0	8.0	201
B	8.0	7.0	7.5	3.75	44
C	21.0	22.0	21.5	10.75	363
D (CONTROL)	0	0	0	0	0

REMEMBER AREA = πr^2

3 CONCLUDE WHICH TYPE OF ANTIBIOTIC WAS THE MOST EFFECTIVE AGAINST BACTERIAL GROWTH

THE MOST EFFECTIVE ANTISEPTIC WAS ANTISEPTIC C BECAUSE IT HAD THE LARGEST CLEAR ZONE AROUND THE DISC WITH AN AREA OF 363 mm^2

Record the diameter of each clear zone to the nearest whole mm, and remember to calculate the area using the radius (taken as half the value of the mean diameter of each zone)

Aseptic techniques

- These techniques are important to use in order to **prevent** the bacterial cultures on the agar plate from **being contaminated** by other micro-organisms or human pathogens from outside

- Contamination will have a **negative impact** on the growth of the bacteria under investigation
- When doing the investigation above, use the following **aseptic techniques**:
  - Keep windows and doors **closed** to prevent air movement
  - **Disinfect** surfaces and utensils regularly to prevent contamination
  - Ensure that you use **sterile equipment** and discard afterwards (especially plastic instruments)
  - Work near a **Bunsen flame** when transferring bacteria to ensure that microbes in the air are drawn away by rising hot air
  - For the same reason as above, hold the **flame** close to the neck of the glass container of the broth every time it's opened or closed

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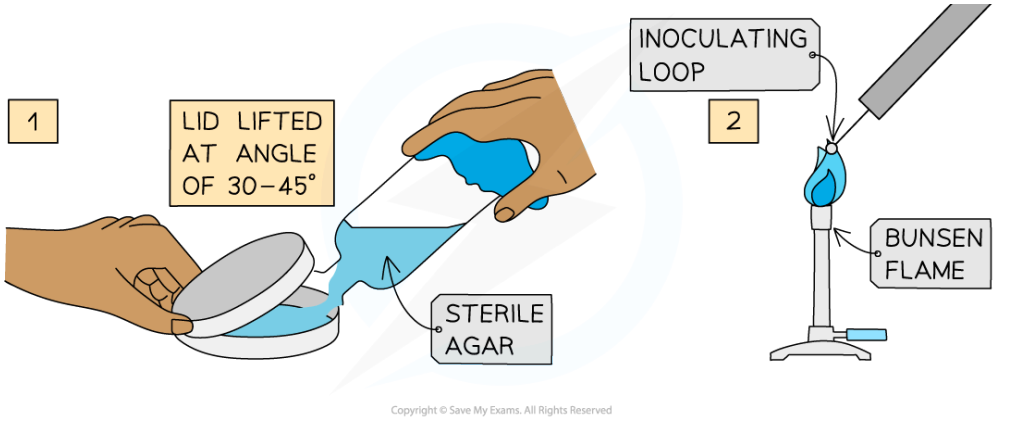

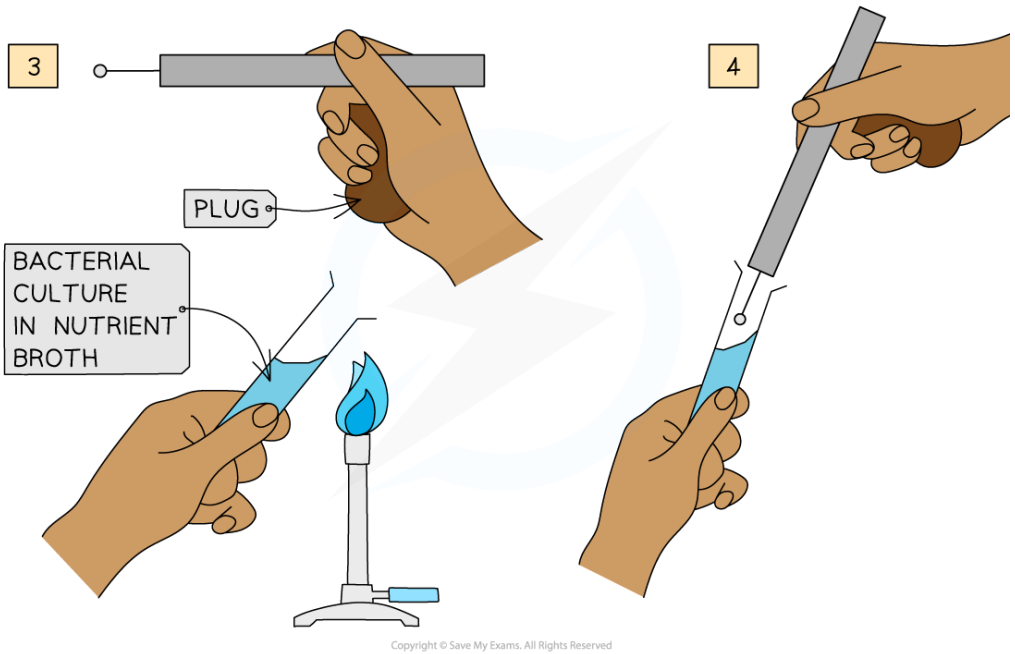

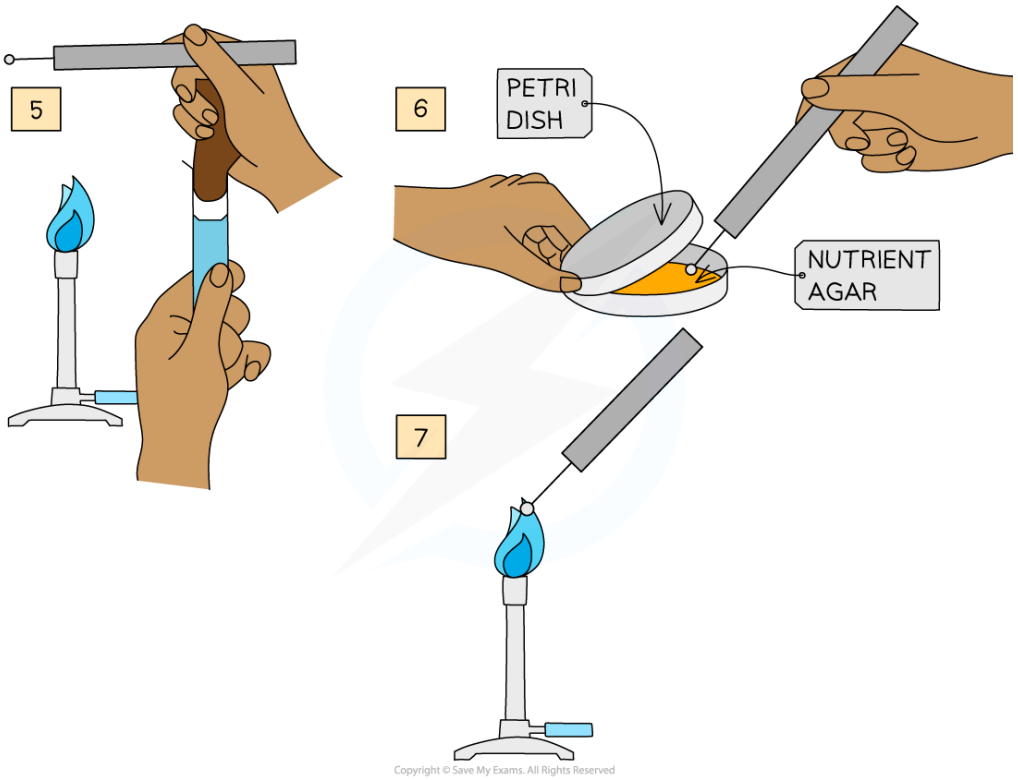

To prepare an uncontaminated culture of micro-organisms, this procedure can be followed

Aseptic Techniques Table

Your notes

STEPS	EXPLANATION
1. WHENEVER WORKING ASEPTICALLY, ALL WORK SHOULD BE CARRIED OUT IN FRONT OF A LIT BUNSEN BURNER WITH A YELLOW FLAME.	THE FLAME CREATES A CONVECTION CURRENT ABOVE THE BENCH, PREVENTING CONTAMINATION OF ANY MICROORGANISMS IN THE AIR.
2. HOT AGAR JELLY IS POURED INTO A STERILISED PETRI DISH. THE AGAR IS LEFT TO COOL AND SET.	THE PETRI DISH AND CULTURE MEDIUM ARE HEATED TO A HIGH TEMPERATURE TO KILL ANY POTENTIAL MICROORGANISMS THAT COULD CONTAMINATE THE EXPERIMENT.
3. AN INOCULATING LOOP IS PASSED THROUGH A HOT FLAME BEFORE IT IS USED TO TRANSFER BACTERIA TO THE CULTURE MEDIUM.	ANY MICROORGANISMS ON THE LOOP ARE KILLED TO PREVENT CONTAMINATION.
4. PETRI DISHES SHOULD ONLY BE OPENED AS LITTLE AS POSSIBLE, AT THE SIDE FACING THE BUNSEN BURNER.	THIS DECREASES THE RISK OF MICROORGANISMS CONTAMINATING THE DISH.
5. THE LID OF THE PETRI DISH SHOULD BE SECURED WITH TAPE AT INTERVALS AROUND THE DISH AND STORED UPSIDE DOWN.	THIS PREVENTS DROPS OF CONDENSATION (ANOTHER SOURCE OF CONTAMINATION) FROM DROPPING ONTO THE SURFACE OF THE AGAR.
6. THE CULTURES SHOULD NOT BE INCUBATED ABOVE 25℃ IN A SCHOOL LABORATORY.	THIS RESTRICTS THE GROWTH OF HARMFUL PATHOGENS (WHICH ARE MORE LIKELY TO GROW AT HIGHER TEMPERATURES).

Your notes

Examiner Tips and Tricks

It is vital that one of the paper discs placed on the bacterial agar plate is not soaked in plant extract but in ethanol instead. This is to ensure that any differences in bacterial growth observed can be attributed to the antimicrobial properties of the plant

extracts used and not some other factor (such as the paper discs themselves or the presence of ethanol, for example)

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Development of Drugs & Drug Testing

- Whenever new drugs are developed, they first need to be **tested** for effectiveness and side-effects, before they can be sold to the general public
- These are known as **clinical trials** and are crucial to avoid exposing the public to drugs that may pose a risk to their health
- Drug testing has not always been this scientific or regulated in the past

Digitalis soup

- **William Withering** was an English scientist from the 1700s
- He is best known for his work on using **digitalis** as a treatment for swelling caused by heart failure (known as **dropsy**)
- Withering treated his patients with an extract made from **foxgloves**, which contains the drug **digitalis**
- Foxglove is **poisonous to humans**, so he made different versions of his remedy containing **different concentrations** of digitalis
- These versions were called his **digitalis soup**
- He experimented on his patients to find the **most effective concentration** of digitalis that would treat dropsy without poisoning the patient

Modern methods of drug testing

- Modern drug testing protocols are much more rigorous than those of the past
- The first step is **modelling** the potential effects of the drug, using **computers**
- Next, the drug will be tested on **human tissues** in a laboratory, before being tested on **animals**
- Should the drug pass these steps without causing any major problems, then it will continue to the **clinical trial** stage where human test subjects will take it
- There are three phases of testing during clinical trials:
  - **Phase 1** involves a small group of healthy individuals to determine how the body will react to the drug, the side effects of the drug and the correct dosage that should be taken
  - **Phase 2** will be done on a larger group of patients (non-healthy individuals who require the drug) to determine the effectiveness of the drug
  - **Phase 3** involves comparing the drug to existing drugs to see if it works any better. A large number of patients are split into two groups, each of which receives either the new drug or the existing one

Placebos

- **Placebos** are typically done during phase 2 of a clinical trial
- Patients are split into two groups - one will receive the drug and the other group will be given a placebo, which **looks exactly like the drug** but contains **no active ingredients**
  - The patients are **not told** which group they are in
- This provides a way for scientists to determine whether the drug actually **works**
- Certain patients display what is known as the **placebo effect**
  - This is where a patient will show improvements in their health due to the **belief** that they are receiving the drug

Double blind studies

- These happen during phase 2 and 3 of clinical trials
- Double blind means that **neither the patient nor the doctor** knows which patient is receiving the drug or the placebo
- This reduces the effect that the attitude of either doctor or patient may have on the results
  - For example, in some cases, a doctor may believe a patient is improving more if they know the patient is receiving the drug

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