

Edexcel B Biology A-Level

Core Practical 3

Make a temporary squash preparation of a root tip to show stages of mitosis in the meristem under the light microscope



Plant cells undergo mitosis at shoot and root tips in areas called **meristems**. Cells in the meristems are **totipotent** and retain the ability to **differentiate**. The **mitotic index** of a sample is the **ratio** of cells **undergoing mitosis** to the **total number of cells in a sample**. To find the **mitotic index**, cells from the meristem must be viewed under an **optical microscope**. The number of cells undergoing the **various stages of the cell cycle** (interphase, prophase, metaphase, anaphase, telophase) can be observed with the optical microscope and the relative length of these different phases calculated.

Equipment

- Optical microscope
- Microscope slides and cover slips
- Water bath (55 °C)
- White tile
- Paper towels
- Stop clock
- Hydrochloric acid (1 mol dm⁻³ HCl)
- Acetic orcein stain
- Distilled water
- Scalpel
- Dissecting needles
- Scissors
- Root tip (garlic)
- Small bottle

Method

1. Heat 1 mol dm⁻³ HCl at **55°C** in a water bath for 15 minutes.
2. Place the garlic clove in the bottle of HCl and leave it for 5 minutes.
1. Cut a small sample of the root tip, **5-10mm in length**, using a **scalpel**.
2. Transfer root tip to **acetic orcein stain** and heat in the water bath for 5 minutes.
3. Place tip on a microscope slide. **Macerate** with a needle to spread the cells out. This makes the chromosomes visible and will therefore show which cells are undergoing mitosis.
4. Lower the cover slip down carefully onto the slide. Make sure there are **no air bubbles** in the slide which may distort the image, and that the coverslip doesn't slide sideways which could damage the chromosomes. Wrap in a paper towel and gently 'squash' the slide.



5. Place under a microscope and set the **objective lens** on the lowest magnification, then use the **coarse adjustment knob** to move the lens down to just above the slide.
6. Use the **fine adjustment knob** to carefully re-adjust the focus until the image is clear (can use a higher magnification if needed).
7. **Count the cells in the various stages of mitosis** and divide this by the total number of cells to calculate the percentage of the total cell cycle that stage represents.
8. To calculate **mitotic index**, cells **undergoing mitosis** must be counted (cells with **chromosomes visible**), as well as the **total number of cells**.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Hydrochloric acid	May cause harm/irritation to eyes or in cuts	Wear eye protection; avoid contact with skin	Wash off skin immediately; flood eye/cuts with cold water	Low
Acetic orcein	May cause harm/irritation to eyes or in cuts	Wear eye protection; avoid contact with skin	Wash off skin immediately; flood eye/cuts with cold water	Low
Scalpel	Cuts from sharp object	Cut away from fingers; use forceps to hold sample whilst cutting	Elevate cuts; apply pressure; seek medical assistance	Low
Broken glass	Cuts from sharp object	Take care when handling slides and coverslips; keep glassware away from edge of desk	Elevate cuts; apply pressure; do not remove glass from wound; seek medical assistance	Low

