

# STANDARD OPERATING PROCEDURE:

## Preparing animal and plant cell slides

*Note: To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment.*

### 1. Introduction

The examination and comparison of plant and animal cells is a hands-on activity suitable for junior and senior secondary science students. Many cells are almost transparent under the microscope and the use of simple stains allows the cells and some of their structures to be easily visible.

A wet mount of an onion membrane is used to represent plant cells and is stained with an iodine stain that reacts with any starch present to produce a blue/black colour. A cell smear prepared from fresh meat purchased from retail stores is used to represent animal cells that are stained with methylene blue stain. Methylene blue is a basic dye that is used to stain animal cells making the nucleus more visible.

Cell type	Specific features	Some common features
Animal Cell	No cell wall Irregular shape One or more small vacuoles	Cell membrane Nucleus Cytoplasm
Plant Cell	Cell wall Fixed shape Chloroplasts Large central vacuole	Mitochondria Ribosomes Endoplasmic reticulum

### 2. Context

- These instructions are for the use of science teachers, technicians and secondary school students who are under the direct supervision of a teacher.
- Do not make up staining solutions for the first time without seeking practical advice from an experienced colleague.

### 3. Safety notes

- Science ASSIST does not recommend the use of cells harvested from humans.
- Only science technicians or teachers should prepare the staining solutions.
- A chemical risk assessment should be performed before preparing solutions of iodine and methylene blue. Avoid breathing dust, vapours or mist. These chemicals are toxic when ingested and can cause skin and eye irritation. Consider if anyone is allergic to iodine.
- Pregnant women should take particular care to avoid exposure to iodine.
- Sensitive individuals may show symptoms of iodism on exposure to small amounts of iodine.

- Students should take care when using glass slides as breakage may occur and small chips can be sharp and cause cuts.
- Ensure students demonstrate responsible behaviour while using sharp knives and other dissecting instruments.

#### **4. Regulations, licences and permits**

None.

#### **5. Equipment**

##### **Onion cell slide**

- Light microscope
- 0.01M Iodine stain in a dropper bottle – teacher or technician to prepare.
- A fresh onion
- Glass microscope slides and No 1 glass or plastic cover slips
- Paper towel or tissue
- Forceps
- PPE including gloves and safety glasses

##### **Animal cell slide**

- Light microscope
- 0.15% Methylene blue stain in a dropper bottle – teacher or technician to prepare.
- Clean sharp knife
- One 2 cm cube of fresh beef or a sheep kidney purchased from a retail store.
- Glass microscope slides and No1 glass or plastic cover slips
- Paper towel or tissue

##### **Personal Protective Equipment (PPE)**

- Gloves and safety glasses

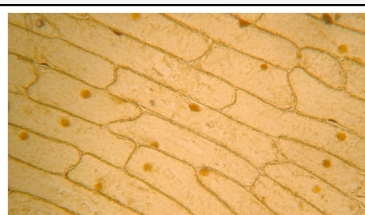
## 6. Operating procedure

### Onion cell slide

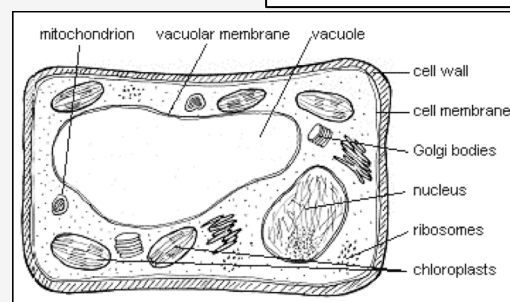
1. Prepare a 0.01M Iodine stain two days prior to class. In an operating fume cupboard or well-ventilated area, weigh 15 g potassium iodide and dissolve in 100 mL of distilled water. Add 3 g iodine crystals and stir to dissolve. Iodine dissolves quickly in concentrated potassium iodide solution. When dissolved, make up to 1 L with distilled water. Store in a dark bottle in a cool area. Label and aliquot into class sets of amber dropper bottles.
2. A teacher or technician should prepare onion sections by cutting the onion into quarters and distributing to students.
3. Divide the onion into its fleshy layers.
4. Using forceps, gently peel back a section of thin epidermis from the concave underside of the layer as shown in Figure 1. Alternatively break the onion layer toward the shiny side and gently peel the two pieces apart revealing the transparent layer of epidermis.
5. Carefully place the epidermis in a **single flat layer** on the centre of a microscope slide. Note that the epidermis is very thin, easy to tear, wrinkle and can fold onto itself.
6. Place 1–2 drops of Iodine stain on the epidermis and spread it out carefully with forceps if folded. Put one edge of a cover slip on the slide to one side of the stain and slowly lower it by means of a mounting needle or toothpick. Make sure there are no air bubbles. See the following references below <sup>1,2,3</sup>.
7. Remove excess stain by touching the edge of the cover slip with a tissue or paper towel.
8. Examine cells under the microscope using the 4x objective. When focused, view at high power (x400 magnification) to make observations of cell size, shape and visible cell structures. Students can draw a labelled diagram.
9. Figure 2 shows a typical plant cell structure.
10. Figure 3 shows onion epidermis stained with Iodine stain at 100x magnification. There are regularly shaped cells lying side by side in a pavement-like arrangement. The cells have a distinct cell wall, cell membrane, nucleus, cytoplasm and vacuole present in the centre of each cell.
11. Remove cover slip and dispose in sharps bin.  
Wash slide in soapy water and rinse well



**Figure 1:** Peeling a section of onion epidermis



**Figure 3:** Onion epidermis at 100x magnification. Iodine stain.



**Figure 2:** Labelled plant cell

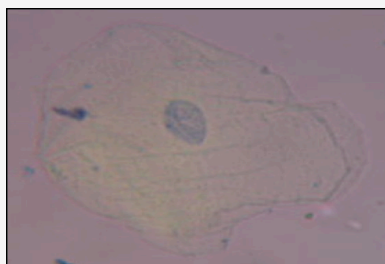
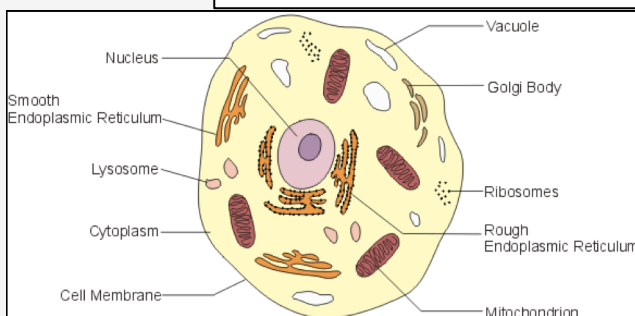
## Animal cell slide

1. Prepare a 0.15% methylene blue stain prior to class. Weigh 1.5g methylene blue powder and dissolve in 100mL of ethanol and make up to 1L with distilled water while stirring. Methylene blue can also be made up in water, however, it dissolves quickly in ethanol. Label and aliquot into class sets of dropper bottles.
2. If using a kidney cut the organ open with a clean sharp knife. Take a glass microscope slide and touch the surface of the cut area with the microscope slide.
3. Add one drop of the methylene blue stain to the microscope slide.
4. Gently place a cover slip over the stained smear by putting one edge of the cover slip on the slide to one side of the stain and slowly lower it by means of a mounting needle or toothpick. Should be free of air bubbles.
5. Remove excess stain by touching the edge of the cover slip with a tissue or paper towel.
6. Examine under the microscope using the 4x objective. When focused, view at high power (x400 magnification) to make observations of cell size, shape and visible cell structures. Students draw a labelled diagram. Look for epithelial cells.
7. If using a piece of red meat cut along the grain line with a clean sharp knife. Take a glass microscope slide and touch the surface of the cut area with the microscope slide. Repeat steps 3-6. This time look for bundles of skeletal muscle fibres and individual fibrils. They are striated in appearance. There may also be some spindle shaped smooth muscle cells from blood vessels with a distinct central nucleus
8. Figure 4 shows a typical animal cell structure.
9. Figure 5 shows an epithelial cell stained with methylene blue stain at 400x magnification. At this magnification, flat, irregular shaped cells with thin cell membrane and nucleus can be seen. The cells do not have a cell wall
10. Figure 6 shows animal cells from a beef sample stained at 400x magnification. At this magnification, striated muscle fibres and some individual fibrils can be seen along with cell walls, cytoplasm and some nuclei.
11. When finished, remove the glass cover slip and dispose in sharps bin. Wash slide in soapy water and rinse well.

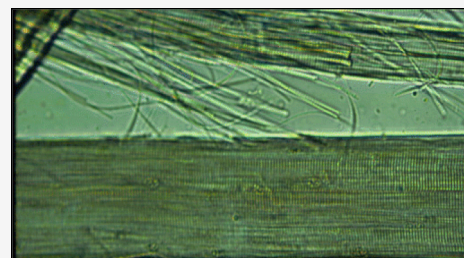
**Figure 4:** Labelled animal cell.

Source:

<http://www.oum.ox.ac.uk/thezone/animals/life/respire1.htm>



**Figure 5:** Cell smear showing an epithelial cell at 400x magnification. Methylene blue



**Figure 6:** Cell smear showing striated muscle fibres at 400x magnification. Source:

[http://www.aps.uoguelph.ca/~swatland/ch5\\_0.htm](http://www.aps.uoguelph.ca/~swatland/ch5_0.htm)

## 7. Trouble shooting/emergencies

- First aid: See latest SDS for more detailed information
  - **If swallowed:** Do not induce vomiting. Rinse mouth with water, and then give water to drink. Seek urgent medical attention.
  - **If in eyes:** Hold open and irrigate with copious quantity of water for at least 15 minutes. Seek medical attention.
  - **If on skin/clothes:** If spilt on skin or clothes quickly wipe off with a dry cloth to absorb as much liquid as possible. Remove contaminated clothes and drench the area with excess water under a safety shower. Seek medical attention.
  - **If inhaled:** Remove to fresh air and seek medical attention if symptoms persist.
  - For further advice contact the Poisons Information Centre on 131 126.

## 8. Waste disposal

- Animal meat should be wrapped and placed in the regular waste for disposal.
- Microscope slides could be washed and reused or disposed of with broken glass, and coverslips should be disposed of with broken glass See Science ASSIST [AIS: Lab glass and porcelain disposal](#)

## 9. Related material

- Manufacturer's Safety Data Sheet
- Science ASSIST [SOP: Use and care of the compound light microscope](#)
- Science ASSIST [AIS: Lab glass and porcelain disposal](#)
- Risk Assessment.

## References:

<sup>1</sup> 'Looking at plant cells (onions)', 4science:educational resources agency website, <http://www.4science.org.uk/assets/files/pdf/02-looking-at-plant-cells.pdf> (Accessed December 2016)

<sup>2</sup> 'Making and staining a wet mount', PowerPoint presentation, Oxford University Press website, [http://lib.oup.com.au/secondary/science/Biology-An-Australian-Perspective/additional\\_resources/practicals/wet\\_mount.ppt](http://lib.oup.com.au/secondary/science/Biology-An-Australian-Perspective/additional_resources/practicals/wet_mount.ppt) (Accessed December 2016)

<sup>3</sup> 'Microscope notes', Living environment course, Mrs. DeBaise webpage, <http://lecoursedebiase.com/Microscope.html> (Accessed December 2016)

Chem-Supply Pty Ltd. *Safety Data Sheet: Potassium iodide*. <https://www.chemsupply.com.au/documents/PA0011CH5J.pdf> (December 2012)

Chem-Supply Pty Ltd. *Safety Data Sheet: Iodine*. <http://chemsupply.customer-self-service.com/images/IL0051CH37.pdf> (September 2015)

Chem-Supply Pty Ltd. *Safety Data Sheet: Methylene blue*. <http://chemsupply.customer-self-service.com/images/ML0451CH4K.pdf> (October 2015)

Dungey, Barbara. 2006. *The Laboratory: a science reference and preparation manual for schools* (Rev. Ed), National Library of Australia: Traralgon, Vic.

Young, J. 2008. *The Biolab Sourcebook*. Triple Helix Resources Ltd, New Zealand