

Victorian Thunderstorm Asthma Pollen Surveillance (VICTAPS) Network

Daily Grass Pollen Count Standard Operating Procedure (Version 1)

Penelope Jones, Ed Newbigin, Edwin Lampugnani and Kia Matley (2017)

Contents

1. Introduction
2. Table of site codes, colours and conversion factors
3. Core daily workflow
4. Preparing slides using the Sylgard adhesive
5. Changing slides in the Burkard monitor
6. Staining and counting slides
7. Grass pollen ID
8. Entering data and completing the pollen forecast
9. Resources for pollen ID
10. Key contacts
11. Acknowledgements

Appendix A: The Burkard air sampler

Appendix B: Preparing Calberla's stain

Appendix C: Protocol for QC counting

1. Introduction

This protocol details the standard operating procedures for use in the Victorian Thunderstorm Asthma Pollen Surveillance Network (VICTAPS) 2017 campaign. The VICTAPS pollen count is funded by the Victorian Government through the Bureau of Meteorology and contributes to the Victorian Department of Health and Human Services' Epidemic Thunderstorm Asthma Forecast Project.

Specifically, VICTAPS will: a) provide a core data input into daily epidemic thunderstorm asthma forecasts generated by the Bureau of Meteorology for the duration of the designated pollen season (October 1st to December 31st); and b) contribute data to pollen and thunderstorm asthma forecasting research.

To support these aims, the VICTAPS pollen count includes two stages of pollen counting:

1. A 'daily count' undertaken daily at each VICTAPS site at 9 am. The aim of this count is to provide a real time surveillance estimate **of grass and total pollen only** in order to support the daily Bureau of Meteorology epidemic thunderstorm asthma forecasts.
2. A 'quality control' (QC) count undertaken retrospectively by designated staff at the University of Melbourne, Parkville. This count aims to both validate the daily counts and to provide additional taxonomic resolution (with a total of 15 taxa counted) for epidemiological and forecasting research.

This document focuses on the protocol for the **daily count**. The protocol for the **QC count** is provided as Appendix C.

2. Table of site codes, colours and conversion factors

	Site colour	Site code	Field No.	Conversion factor*
Hamilton	Green	HML	20	0.972
Creswick	White	CRS	22	0.883
Bendigo	Yellow	BDG	18	1.079
Dookie	Pink	DKE	18	1.079
Churchill	Pink	CHC	20	0.972
Parkville	Light blue	PRK	22	0.883

*Note that each sites' conversion factor has been calculated based on the field of view of the microscope designated for counting at that site. It also assumes that pollen is counted using the **40x objective**. *It is therefore important that VICTAPS counting is always undertaken using the 40x objective and using the designated VICTAPS microscope.*

3. Core Daily Workflow - VICTAPS Pollen Count 2017

1. Change the microscope slide in the Burkard monitor at **9 am** each day. The next day's slide should be labelled and pre-coated with the Sylgard adhesive. Ensure that the sticky side of the new slide faces outwards and that the slide is returned to the start (bottom) position.
2. When in the microscope room, mark the label of the slide just collected (in pencil, not ink) with the date (YYYY/MM/DD), and site code (see table in Section 2).
3. Stain sticky side with **3 drops of Calberla's stain** and carefully cover with coverslip. Press down gently to spread the stain, being careful not to move the cover slip. Seal cover slip to slide with nail polish and allow to dry (~5 min).
4. View slide with the light microscope. You are required to count pollen using the **40x objective** but you may find it easier to focus at 10x or 20x and then switch to the 40x objective.
5. Count **two horizontal transects at 40 x magnification** by scanning across slide from left to right. Aim for the transects to be approximately 1/3 and 2/3 of the way down the slide. Avoid the edges as the pollen distribution here is often poor.
6. Keep track of two counts:
 - a. Grass pollen
 - b. All other pollen
7. Note the final **raw counts** in the red count book (**summing** the counts of the two transects).
8. Convert the raw counts to concentrations (in grains/m⁻³ of air) using the conversion factor specified for your site (see table in Section 2).
9. Enter the data using the data submission portal **ensuring that you enter the converted concentrations, not the raw counts**.
10. Complete the pollen forecast – **use the recommended forecast as a default**.
11. Data must be entered and the forecasts made by **10:30 am***.



***If there are any problems** with making this deadline contact Ed Newbigin on 0428 320 806 or Ed Lampugnani on 0438 564 330

4. Preparing slides using the Sylgard adhesive

Materials

- Sylgard® 527 parts a and b silicone dielectric gel
- Standard glass microscope slides (76.2×25.4 mm approximately, 1.0-1.2 mm thick)
- Tape in the appropriate colour for your site (see table below)

The Sylgard adhesive is easy to prepare, does not interact with the dyes used in pollen counting and is not sensitive to the changes in temperature and humidity likely in Victoria.

Method

Safety note: Read the MSDS for this product. Sylgard® 527 is considered a non-hazardous substance

1. Measure out equal parts of Sylgard parts a and b.
2. Pour the contents into a plastic dish or cup. Mix together using a glass rod or clean pipette. Wipe the rod clean immediately as the glue becomes stickier with time.
3. Wipe the slides clean with a kimwipe.
4. Place a small (~1.5 cm) portion of coloured tape at the bottom of the non-sticky side. This will become the label where you write the site and date of the sample.
4. Spread the glue over the slide using the edge of a microscope slide or a plastic pot label/similar as a spreader. Ensure that the glue is distributed evenly as a thin coat over the middle of the slide.
5. Dozens of slides can be prepared at the same time and stored flat for later use.

	Site colour	Site code
Hamilton	Green	HML
Creswick	White	CRS
Bendigo	Yellow	BDG
Dookie	Pink	DKE
Churchill	Pink	CHC
Parkville	Light blue	PRK

5. Changing slides in the Burkard monitor

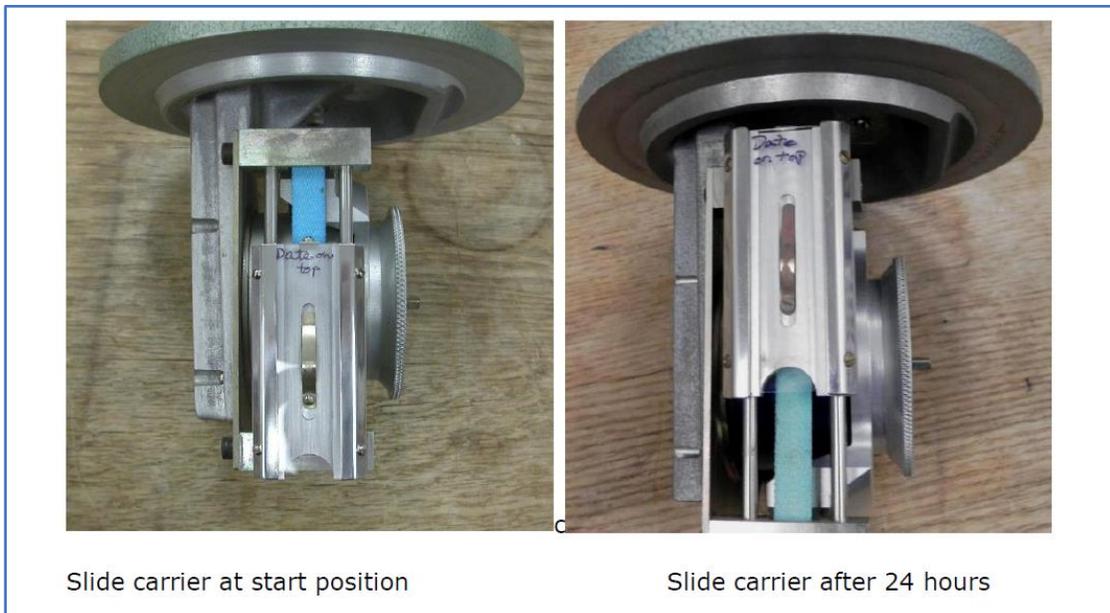
Materials

- Microscope slide pre-coated with the Sylgard adhesive

Method

1. Stop trap from rotating by fixing the drum in place with the pin.
2. Slide lever across and pull out head assembly using the handle.
3. Wind the glass slide back to the start (bottom) position
4. Pull out the slide and place in the slide box.
5. Put new slide in place. The label (coloured tape) should be at the top.
6. Re-wind the clockwork motor using the key (do not overwind – if turned daily, 1-2 times will suffice).
7. **Make sure that the sticky side of the new slide faces the inlet (outwards) and that the slide is in the start (bottom) position (see figure below).**
8. Replace the head assembly in the drum and fix in place with lever.
9. Remove the pin and make sure trap can rotate.

Note that a video of this procedure is available on the online training site



6. Staining and counting slides

Materials

- Calberla's stain
- Glass coverslip (20×60 mm)
- Nail polish
- Compound microscope with 40× objective

Method

1. Mark the label (in pencil, not ink) with the date (YYYY/MM/DD) and site code.
2. Stain sticky side with 3 drops of Calberla's stain and carefully cover with coverslip. Press down gently to spread the stain, being careful not to move the cover slip. Seal cover slip to slide with nail polish and allow to dry (~5 min).
3. After ~5 min, view slide with the light microscope. You are required to count pollen using the **40x objective** but you may find it easier to focus at 10 or 20x and then switch to the 40x objective.
4. **Count two longitudinal transects with the 40 x objective** by scanning across slide from left to right. Count each field of view as you go. Aim for your transects to be approximately 1/3 and 2/3 of the way down the slide. Avoid the edges as the pollen distribution here is often poor.



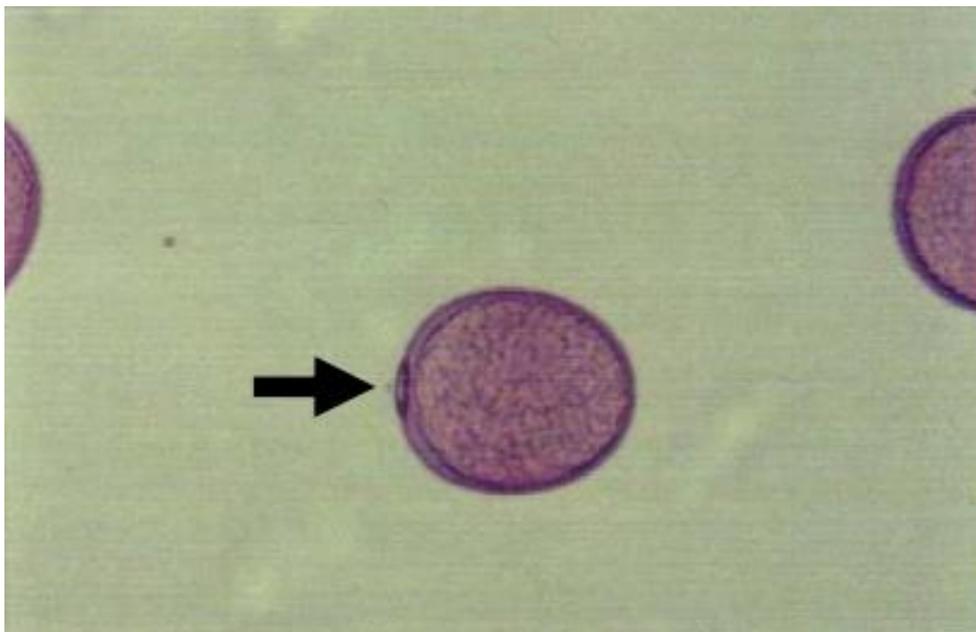
5. Keep track of: **a) grass pollen grains; and b) all other pollen grains**. These will be summed together to give the total pollen count. Use the identification resources to remind you what grass pollen looks like. Take particular care not to confuse grass pollen with *Fraxinus* (ash) and *Plantago* (plantain). **Adjusting the fine focus to see the single pore is a good way of confirming the identity of a grain.**
6. Note down the **raw counts** in the red count book.
7. Convert the **raw counts to grains/m³** using the conversion factor specified (see table in Section 2). Enter the converted counts into the data portal (see Section 8 for data entry protocol).

7. Grass pollen identification

Grass pollen is the one of the most significant outdoor allergens in Australia and the target of the Victorian Thunderstorm Asthma Pollen Surveillance campaign. Most of Melbourne's grass pollen comes from perennial ryegrass (*Lolium perenne*) pastures to the north and west. Many parts of regional Victoria will also have high levels of grass pollen due to surrounding pasture.

Grass Pollen Morphology

Grass pollen is characterised by a **single pore** as indicated by the arrow below.



Other characteristics of grass pollen are:

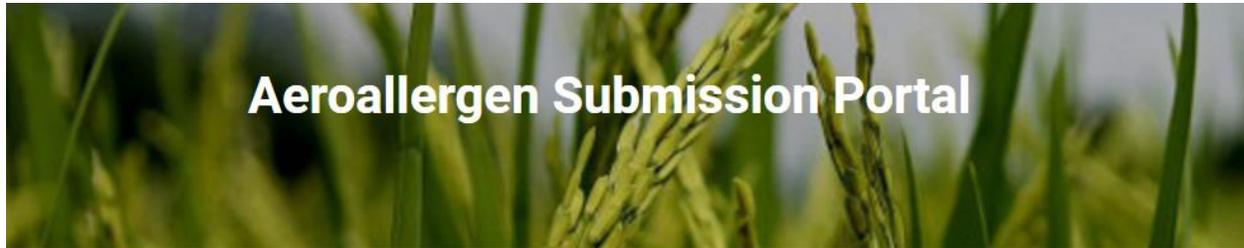
- Overall shape is a slightly flattened circle/ shortened egg shape
- Normally 30-40 μm in size
- Smooth surface texture (no texture/line patterning on the cell wall)
- Markedly granular cell interior (representing starch granules)

Take care to remember that **pollen is 3D** and you may see the pore from different angles. You may also mistake pollen from other taxa (e.g. *Plantago*) if you do not look carefully for more than one pore. **It is important to use the fine focus on the microscope to check your IDs.**

Refer to the 'Grass Pollen Image Collection' on the online training resources for multiple images of grass pollen from numerous angles. Images of other common Victorian pollen types are also available.

8. Entering data and completing the pollen forecast

1. Login to website (<https://submit.nixdev.unimelb.edu.au/>) using your personal login details



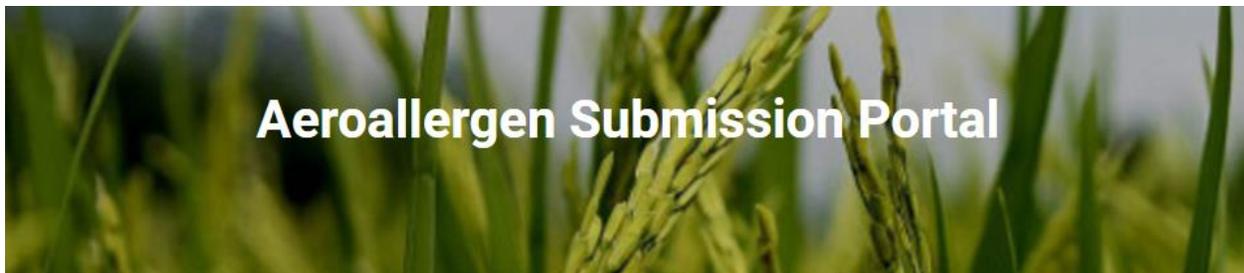
Welcome to the portal!

Username

Password

Login

2. Click on 'Submit Pollen Forecast'



Aeroallergen Submission Portal

Pollen Submission

Hi Penny, welcome to pollen submission!

[Submit Pollen Forecast](#)

[See Submissions](#)

[Logout](#)

3. Enter the site, date and time of slide collection (the latter should be 9:00 am).

Select whether the pollen count is available. **If not (e.g. monitor breakdown, OHS risk due to extreme weather), you must note why in the comments and contact Ed Newbigin on 0428 320 806 or Ed Lampugnani on 0438 564 330.**

Enter the day's pollen count for:

- Grass pollen
- All other pollen

The website will **auto-calculate** the sum of these two to give the **total pollen count**.

****IMPORTANT: Before data entry you MUST convert raw counts to pollen concentrations (grains/m⁻³)**

To do this multiply the raw count by the conversion factor for your site as per the table below.

	Site colour	Site code	Field of View	Conversion factor
Hamilton	Green	HML	20	0.972
Creswick	White	CRS	22	0.883
Bendigo	Yellow	BDG	18	1.079
Dookie	Pink	DKE	18	1.079
Churchill	Pink	CHC	20	0.972
Parkville	Light blue	PRK	22	0.883

Today's Pollen Count

Select Trap Location	Hamilton
Date of Collection	Sunday, 24 September 2017
Time of Collection	09:48
Is the pollen count available?	Yes <input checked="" type="checkbox"/>
Grass Pollen Count	
Other Pollen Count	
Total Pollen Count	0
Select Pollen Level	----
Comments (if any)	

4. Select the appropriate pollen level in the drop-down box based on the **grass pollen count expressed in grains/m³**, following the guidelines in the table below.

Add any relevant comments (e.g. issues with the monitor, unusual observations, anything notable)

Grass pollen (grains/m ³)	Pollen level
0-19	Low
20-49	Moderate
50-99	High
≥100	Extreme

Today's Pollen Count

Select Trap Location	Hamilton
Date of Collection	Sunday, 24 September 2017
Time of Collection	09:48
Is the pollen count available?	Yes <input checked="" type="checkbox"/>
Grass Pollen Count	
Other Pollen Count	
Total Pollen Count	0
Select Pollen Level	----
Comments (if any)	

5. Complete the (grass) pollen forecast for the next 7 days based on the observed grass pollen count and the weather forecast.

Use the suggested pollen forecast as the default. This is based on a statistical model developed by the University of Melbourne.

Pollen Forecast

Sunday, 24 September 2017

Shower or two. Windy.

Partly cloudy. Medium (50%) chance of showers. Winds northwesterly 30 to 40 km/h tending westerly 25 to 30 km/h in the late evening.

 Max °C
Min °C



50 % probability
Likely:

Suggested Pollen Forecast

Select Pollen Forecast

6. Hit submit. Data must be submitted **no later than 10:30 am.**

Friday, 29 September 2017

A few showers.

Cloudy. High (70%) chance of showers. Winds northwesterly 20 to 30 km/h tending westerly during the day.

 Max 14 °C
Min 5 °C



70 % probability
Likely: 1 to 3 mm

Suggested Pollen Forecast

Select Pollen Forecast

SUBMIT

7. Confirm that your data entry is correct.

Hit save.

If for any reason you realise you have made an error with your data entry it is important that you contact Ed Lampugnani on 0438 564 330.



[Home](#)

Pollen Count

Please review your data before submitting:

Location	Melbourne
Date and time	2017-06-27 16:50
Was the pollen count available?	Yes
Grass Pollen Count	20
Other Pollen Count	20
Total Pollen Count	40
Level	L
Remarks	dummy

Forecasts

SAVE

9. Resources for Pollen ID

The following are useful and reliable resources for pollen ID.

Note that to use these you will need to either:

- Know the family/genus/taxon name that you want to look up; or
- Know the shape (e.g. you can filter or search by shape in some databases)

There are also a collection of pdf resources on the online website.

Pollenwarndienst: <https://www.pollenwarndienst.at/en/aerobiology/pollen-atlas.html>

Global pollen project: <https://globalpollenproject.org/>

Pollen wiki (European): <https://pollen.tstebler.ch/>

Australian Pollen and Spore Atlas: <http://apsa.anu.edu.au/>

NSW pollen collection: http://www.geo.arizona.edu/palynology/nsw/search_graphics_sketch.htm

10. VICTAPS Key Contacts 2017

If you have any issues, please contact the appropriate person as shown in the table below. Where two people are listed try the first listed person first.

Issue			
Missed pollen reading	Edwin Lampugnani Ed Newbiggin	edwin.lampugnani@unimelb.edu.au edwardjn@unimelb.edu.au	0438 564 330 (03) 8344 4871 0428 320 806
Data entry system	Edwin Lampugnani	edwin.lampugnani@unimelb.edu.au	0438 564 330
Other technical issues	Edwin Lampugnani	edwin.lampugnani@unimelb.edu.au	0438 564 330
General project management issues	Edwin Lampugnani Ed Newbiggin	edwin.lampugnani@unimelb.edu.au edwardjn@unimelb.edu.au	0438 564 330 (03) 8344 4871 0428 320 806
Pollen ID	Penny Jones Kia Matley	Penelope.Jones@utas.edu.au Kia.Matley@gmail.com	0490 389 415 0431 274 428
Supplies	Edwin Lampugnani Kia Matley	edwin.lampugnani@unimelb.edu.au Kia.Matley@gmail.com	0438 5664 330 0431 274 428

11. Acknowledgements

The VICTAPS project is funded by the Victorian Government. Its implementation is being led by the University of Melbourne in partnership with the Bureau of Meteorology. The University of Tasmania has contributed towards training and instrumentation.

This protocol has been modified from the Melbourne Pollen Count Manual and the AirRater Project Standard Protocol, with reference to the Interim Australian Airborne Pollen And Spore Monitoring Standard And Protocols (Beggs PJ, Davies JM and Milic A 2017). The latter has been prepared by the authors in consultation with investigators and collaborators of the Australian Pollen Allergen Partnership including: Professor Connie Katelaris (Campbelltown Hospital), Ms Pamela Burton (Campbelltown Hospital), Dr Danielle Medek (Waitemata District Health Board, New Zealand), Associate Professor Edward Newbiggin (The University of Melbourne), Professor Simon Haberle (The Australian National University), Dr Edwin Lampugnani (The University of Melbourne) and Dr Jane Al Kouba (Macquarie University), Professor Fay Johnston (University of Tasmania), Dr Penelope Jones (University of Tasmania), Associate Professor Cenk Suphioglu (Deakin University) and Dr Philip Taylor (Deakin University).

The following institutions are acknowledged for their hosting of monitoring sites:

- The University of Melbourne (Parkville, Dookie, Creswick)
- Latrobe University (Bendigo)
- Federation University (Churchill)
- Western District Health Service (Hamilton)
- Deakin University (Warun Ponds, Burwood).

Appendix A: The Burkard air sampler

Air sampling is routinely used to monitor airborne particles such as pollen and spores. The VICTAPS network uses Burkard volumetric samplers (see Figure 1 below). These samplers are the standard of reference for pollen counters world-wide.

Burkard monitors have a built-in vacuum pump that draws air in at a fixed rate (10 L min^{-1}) through a narrow inlet. This approximates the normal human breathing rate. The device freely rotates around a vertical axis and a weather vane ensures the inlet always points into the wind. Particles are captured on an adhesive-coated surface that is slowly moved past the inlet by a clockwork-driven drum. The instrument is relatively reliable, lightweight, corrosion resistant and easy to operate and maintain.

An advantage of the Burkard instrument is its ability to record continuously, either over a 24 hour period (slide mode) or 7 day period (tape mode). The VICTAPS network uses Burkard monitors in slide mode as shown in Figure 2 below. In this case a microscope slide is held within the instrument head. The slide is moved slowly by clockwork motor over a 24 hour period to generate a daily sample.



Figure 1: A Burkard volumetric sampler. The samplers are elevated from the ground.



Figure 2: The interior of the head of the Burkard monitor for 24-hour sampling. The microscope slide faces the air inlet and is moved gradually over the 24-hour period by the clockwork motor.

Appendix B: Preparing Calberla's stain for pollen staining

Calberla's stain contains basic fuchsin and stains pollen grains pink, but does not stain fungal spores or most of the other debris usually present on slides. The dye is deposited on the outer wall of pollen grains, enhancing the surface characteristics necessary for identification. Some suppliers sell Calberla's in a ready-to-use form however it is easy to prepare using the protocol below.

Calberla's stain will be prepared by staff at the University of Melbourne Parkville and supplied to all other VICTAPS sites.

Materials

- 5 ml Glycerol
- 10 ml Ethanol 95% (v/v)
- 15 ml Distilled water
- Saturated aqueous basic fuchsin*
- Glycerol

Method

1. Mix together a 50:50 solution of saturated aqueous basic fuchsin and glycerol. Upon cooling the solution will form a dark pink gel.
2. Store in an airtight jar for future use.
3. Mix the glycerine, ethanol, distilled water and 6 drops of the pre-prepared fuchsin/glycerol solution. Store the Calberla's solution in a clear bottle with a dropper lid.

** Basic fuchsin is available in powder form from a number of suppliers. Only a very small amount is needed to make up the saturated aqueous basic fuchsin.*

Appendix C: Protocol for Quality Control (QC) counting

This protocol details the counting method to be undertaken by designated QC pollen counters at Parkville, University of Melbourne. All VICTAPS slides will be sent weekly to Parkville, where they will be re-counted by the QC counters according to this protocol. The QC counts aim to: a) validate the daily grass/total pollen counts; and b) to provide additional taxonomic resolution.

Materials

- Compound microscope with 40× objective

Method

1. Write the date (YYYY/MM/DD) and site code at the top of the count sheet.
2. View slide with the light microscope. You are required to count pollen using the **40x objective** but you may find it easier to focus at 10 or 20x and then switch to the 40x objective.
3. **Count 4 longitudinal transects with the 40 x objective** by scanning across slide from left to right. Count each field of view as you go. Aim for your transects to be approximately evenly spaced down the slide. Avoid the edges as the pollen distribution here is often poor.



4. Using the count sheets provided, keep track of the taxa listed in the table overleaf. Use the identification charts to confirm the identity of the grains. Any pollen that does not belong to any of these taxa (or which is un-identifiable) should be counted as 'other'. Adjusting the fine focus is a good way of making sure you have seen all pores and colpi.
5. Tally the pollen counts for each taxon. **Note that *Alternaria* is a fungal spore and is therefore not to be included in the total pollen count.**
6. Note down the **raw counts** in the red count book.
7. Convert the raw counts to grains/m⁻³ using the conversion factor specified for your microscope. Enter into the data submission portal.

Taxa for QC counting – VICTAPS 2017

Scientific name	Common name	Likely dominants within pollen taxon (for guidance only)
<i>Acacia</i> (t)	Wattle	n/a
<i>Alternaria</i> ** (f)	Alternaria fungus	n/a
Asteraceae (w)	Daisy family	n/a
<i>Betula</i> (t)	Birch	<i>Betula pendula</i>
Brassicaceae (w)	Brassica family	<i>Brassica rapa/Brassica napus</i>
<i>Allocasuarina</i> (t)	Casuarina	n/a
Cupressaceae (t)	Cypress family	<i>Callitris</i> spp., <i>Cupressus</i> spp.
Myrtaceae (t)	Eucalypt family	<i>Eucalyptus</i> spp.
Oleaceae (t)	Olive family	<i>Olea europaea</i> , <i>Fraxinus</i> spp.
Pinaceae (t)	Pine family	<i>Pinus</i> spp.
<i>Plantago</i> (w)	Plantain	<i>Plantago lanceolata</i>
Platanaceae (t)	Plane tree family	<i>Platanus</i> spp.
Poaceae (g)	Grass family	n/a
Ulmaceae (t)	Elm family	<i>Ulmus</i> spp.
Urticaceae (w)	Nettle family	<i>Parietaria</i> spp, <i>Urtica</i> spp.
Other/Unknown	All other pollen grains	n/a
Total		

t=tree, g=grass, w=weed, f=fungal spore

** Note that *Alternaria* is a fungal spore and is therefore NOT included in the total pollen count