Australian Airborne Pollen and Spore Monitoring Network Interim Standard and Protocols

Version 2, 14 September 2018

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1 Executive Summary

Despite the high prevalence of allergic rhinitis and asthma, Australia is one of the few developed countries without a standardised national airborne pollen and spore monitoring program. The growing public demand for an Australian Airborne Pollen and Spore Monitoring Network prompted the establishment of a standardised methodology for pollen and spore monitoring that can be applied by existing and newly created Australian monitoring sites. This Standard and Protocols document is designed to articulate best practice for pollen and spore monitoring that can be adopted and implemented nationally by all current and future aerobiology research projects as well as for Australian monitoring and forecasting services. It has been written both for the National Health and Medical Research Council AusPollen Partnership Project entitled “AusPollen: Implementation of a standardized national pollen alert system for better management of allergic respiratory health” and as part of the Victorian Department of Health and Human Services’ thunderstorm asthma forecasting development program which is a collaboration between the Australian Bureau of Meteorology and a series of academic partner institutes.

The Standard specifies principles, protocols and procedures to guide monitoring of airborne pollen and spores including all aspects of sample collection, processing, counting and reporting. The overall objective is to enable generation of quality data on exposure to allergenic pollen and fungal spores that can be used to enhance the quality of life of individuals with allergic diseases, assist with clinical diagnosis and management of patients and inform public health policy and practices. This Standard and Protocols document is designed to improve the consistency of processes between sites and to enhance reliability of data enabling better comparability of data derived from different locations across the continent. This is the first Standard and Protocols for Pollen and Spore Monitoring that has been written specifically for Australia and this serves as an interim document that will be amended and adapted according to the experience of users and expert review annually over the next three years and beyond.

Throughout the document the rationale for requirements and recommendations for best practice is described. The document outlines minimum standards that should be adopted by all sites. All sites are encouraged to be compliant with the minimum standards within one year of release of this document and to adopt best practice. Individual projects or organisations that are responsible for management of particular pollen monitoring sites may utilize the Audit process (26.9 Pollen Monitoring Site Audit Checklist) to evaluate compliance of individual sites to the minimum requirements outlined within this Standard and Protocol document and to implement audit report recommendations. Protocols designed specifically for surveillance of grass and total pollen concentrations for the purposes of development of daily thunderstorm asthma pollen forecasting are also provided. The summary of minimum requirements suggested by this Standard are listed in Table 1.

Table 1: Summary of the minimum essential/required aspects of the Standard and Protocols.

Aspect	Minimum requirement
Sampler and Position	A Hirst-type, continuous flow volumetric sampler must be placed on a readily accessible, flat, horizontal surface, following the minimum location criteria: • Height above the ground: 1.5 – 15 m; • Minimum 1m vertical and 2m horizontal distance from any protruding supporting structures; • Sampling inlet with minimum clear sky angle of 120°; • Unrestricted airflow of 270° around the sampling inlet orifice.
Flow Rate	Continuous sampling with flow rate of 10 L/min (checked weekly).
Monitoring Period	Minimum: 1 October - 31 December in temperate regions (e.g., Melbourne) and 1 November – 31 March in subtropical regions (e.g., Sydney, Canberra and Brisbane). Highly recommended: continuous monitoring all year round.
Slide Changeover Time	At the same chosen time each day or week. To enable sampling, counting and data entry for forecasting purposes the ideal daily sampling time is between 7 and 11am.
Adhesive	Silicone (polydimethilsiloxane) based adhesive is recommended.
Mounting Media with Stain	Minimum: Mounted with glycerine jelly, gelatine or polyvinyl alcohol (e.g., Gelvatol). Highly recommended: To enhance visualisation by light microscopy staining of pollen with Fuchsin.
Surface Examined	Minimum: 10% of the whole deposition area which can be achieved with 3 longitudinal (horizontal) transects. Highly recommended: 4 longitudinal transects to capture good representation of taxa that are present in low concentrations.
Counting Magnification	40x objective and 10x ocular lens (400x magnification).
Conversion Factor	Atmospheric pollen or fungal spore concentrations should be expressed as the daily average pollen grains or fungal spores per cubic meter of air. This can be calculated using the following equation, which takes into account all the relevant factors. $$[\text{Pollen or Spore}] = n\left[\frac{L \times W}{L \times (\frac{F}{M}) \times N} \times \frac{1}{D \div 1000 \times t}\right]$$ where

	[Pollen or Spore] - atmospheric pollen grain or fungal spore concentration per cubic metre n - number of pollen grains or fungal spores counted in the analysed area of the microscope slide L - length of impaction area on the melinex tape or glass slide (mm) e.g., 48 mm W - width of impaction area on the melinex tape or glass slide (mm) e.g., 14 mm F - field number written on the eyepiece (typically a number between 18 mm and 25 mm) M - objective magnification (typically 40) N - number of transects e.g., 4 D - flow rate of the equipment (l/min) e.g., 10 l/min t - duration of the sampling period (min) e.g., 1440 min
Taxa Reported	Minimum: Grass and total pollen. Highly recommended: counting and reporting of locally abundant and relevant (allergenic) pollen and spores (examples in Tables 5 and 6).

2 Acknowledgements

Expert Consultation Group (Reference Group)

This Australian Airborne Pollen and Spore Monitoring Network Standard and Protocols document has been prepared in consultation with investigators and collaborators of the Australian Pollen Allergen Partnership whose experience and insights has provided valuable direction and detail upon which the contents have been developed. In particular, we thank collaborators and the expert Reference Group who have provided feedback on this document: AusPollen Partnership Investigators Dr Danielle Medek (Waitemata District Health Board, New Zealand), Dr Elizabeth Ebert (Bureau of Meteorology), Dr Edwin Lampugnani (The University of Melbourne), Associate Professor Cenk Suphioglu (Deakin University) and Dr Jane Al Kouba (Macquarie University). We also thank AusPollen Partnership Investigators Dr Bircan Erbas, Professor Alfredo Huete and Dr Rieks van Klinken for their ongoing valuable contribution to the formulation and implementation of the standardized national pollen monitoring system.

We specifically acknowledge the contributions of Dr Andre Zerger and Chacko Thomas (Bureau of Meteorology) and Dr Edwin Lampugnani for assistance with developing plans for the inclusion of pollen monitoring site metadata within the National Environmental Monitoring Sites Register, and Dr Edwin Lampugnani and Dr Elizabeth Ebert for their assistance in developing plans for inclusion of pollen data within the National Computational Infrastructure repository.

International Collaborators

We are thankful to international collaborators who responded to questions about aspects of pollen and spore monitoring: Dr Bernard Clot (MeteoSwiss, Switzerland) and Professor Dr Jeroen Buters (Technical University of Munich, Germany).

Funding

We acknowledge funding that has contributed to support for the preparation of this document from the National Health and Medical Research Council and matching co-contributions from partner organisations including the Australasian Society of Clinical Immunology and Allergy, Asthma Australia, Bureau of Meteorology, Commonwealth Scientific and Industrial Research Organisation, Stallergenes Australia and Federal Office of Meteorology and Climatology MeteoSwiss (Switzerland). We are also thankful to Victorian Department of Health and Human Services and the Australian Bureau of Meteorology for funding that supported the preparation of the document.

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5 List of Acronyms and Abbreviations

AAA	Australasian Aerobiology Association
AAAAI	American Academy of Allergy, Asthma & Immunology
ACEAS	Australian Centre for Ecological Analysis and Synthesis
AirRater	AirRater Airborne environment and respiratory symptom tracking system [University of Tasmania]
ASCIA	Australasian Society of Clinical Immunology and Allergy
AusPollen	Australian Pollen Allergen Partnership
AusPollen Aerobiology Collaboration Network	Extended network associated with the AusPollen Partnership including the ARC Discovery project (DP1700101630), AirRater and VicTAPS
BoM	Bureau of Meteorology [Australia]
CC-BY	Creative Commons Attribution
CSIRO	Commonwealth Scientific and Industrial Research Organisation [Australia]
DSITI	Department of Science, Information Technology and Innovation [Queensland]
EAN	European Aeroallergen Network
EAS	European Aerobiology Society
EPA	Environment Protection Authority
IAA	International Association for Aerobiology
ICMJE	International Committee of Medical Journal Editors
IPNI	International Plant Names Index
NAB	National Allergy Bureau [US]
NATA	National Association of Testing Authorities [Australia]
NEII	National Environmental Information Infrastructure [Australia]
NEMSR	National Environmental Monitoring Sites Register [Australia]
NHMRC	National Health and Medical Research Council [Australia]
OAIS	Open Archival Information System
PPE	Personal Protective Equipment
TERN	Terrestrial Ecosystem Research Network [Australia]
TUM	Technical University of Munich [Germany]
VicTAPS	Victorian Thunderstorm Asthma Pollen Surveillance
WAO	World Allergy Organization
WHS	Work Health and Safety

6 List of Terms and Definitions

Adapted from European Committee for Standardization Technical Specification.

Accuracy - closeness of agreement between a measured quantity value and a true quantity value of a measurement

Clockwork system - mechanism with a spring and toothed gearwheels, used to drive a mechanical clock, toy, or other device

Drum - cylindrical device for the mounting of a plastic tape coated with adhesive

Exhaust port – opening at the bottom of the sampling chamber (above the vacuum pump)

Flow meter - instrument for measuring the flow rate of a fluid (e.g., air)

Flow rate - amount of fluid (e.g., air) that flows through the orifice in a given unit of time

Fume hood - ventilation device that is designed to limit exposure to hazardous or toxic fumes, vapours, gases or dusts

Fungal spore (or spore) - reproductive unit capable of giving rise to a new individual with or without sexual fusion

Magnification - magnifying power of an instrument

Microscope - optical instrument having a magnifying lens or a combination of lenses for inspecting objects too small to be seen or too small to be seen distinctly and in detail by the unaided eye

Orifice - opening or aperture; a slot-like opening on the side of the trap

Pollen (or pollen grain) - male gametophyte of seed plants, consisting of microscopic grain discharged from the anthers (Angiosperms) or from a male cone (Gymnosperms)

Precision - closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Repeatability- condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time

Reproducibility - condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects

Sensitivity - in aerobiology, measurement of the proportion of search particle which is correctly identified

Site (monitoring site) - a geographic location where pollen and fungal spore monitoring is performed

Slide - rectangular piece of glass on which an object is mounted or placed for examination under a microscope

Specificity - in aerobiology, measurement of the proportion of non-searched particles which are correctly identified as different from the searched particles

Trap (also known as sampler) - container or device used to collect something

Vacuum pump - pump or device by which a partial vacuum can be produced

Wind vane - mechanical device attached to an elevated structure; rotates freely depending on the direction of the wind

7 Introduction / Context / Rationale

Airborne pollen and fungal spores are biological particles that have an unquestioned impact on human health, inducing a wide diversity of allergic reactions and diseases in susceptible members of our community with specific allergic sensitivities to these aeroallergen sources. Insight into local aerobiology has been demonstrated to facilitate building preventive measures including self-management of and professional help for allergy symptoms of the susceptible individuals, reducing the burden and cost to the health sector of allergic respiratory disease and helping in the establishment of a short-term pollen forecasting network (Beggs et al. 2015). An essential part of managing the pollen and spore[1] monitoring is conducting it in a standardised way.

7.1 Purpose of Standardised Pollen and Spore Monitoring

There are many reasons for and benefits of standardisation of Australia's pollen and spore monitoring and related activities and services. Standardisation should result in more-accurate and precise (i.e., correct) data and information regarding ambient concentrations of pollen and fungal spores in Australia. Standardisation should also enhance intrastate, interstate, and international comparison of Australia's pollen and spore data, leading to a better understanding of our environmental exposures to allergenic bioaerosols and enhancing broadly multi-disciplinary research capacity. Pollen and spore monitoring information is a highly useful resource for public health and emergency services for managing the timing of medication use and implementation of preventive measures. Moreover, individuals suffering from pollen allergies can organise their outdoor activities in a timely fashion so as to avoid likely danger periods. The information on airborne pollen and fungal spore data should be also valuable for a wide range of other disciplines and applications and will be a valuable resource for tracking changes attributed to climate change.

The existing "Aeroallergen Monitoring Standard for The Asia Pacific Region" (Hasnain et al. 2007) is now a decade old. Based on the literature cited in it (all but four of its references being pre-2000), it is largely not based on relevant Australian or international research conducted over the past almost 20 years. The 21 November 2016 Melbourne thunderstorm asthma event has further strengthened state and national need for enhanced quantity and quality of pollen and spore monitoring (Inspector-General for Emergency Management 2017). This is the first Standard and Protocols document for Pollen and Spore Monitoring that has been written specifically for Australia. It serves as an interim document that will be amended according to the experience of users and ongoing expert review.

As part of the Australian Centre for Ecological Analysis and Synthesis (ACEAS 2016) which was a Facility of Australia's Terrestrial Ecosystem Research Network (TERN) from 2009-2014, the Australian Aerobiology Working Group was established in 2013 and has collated, analysed and synthesised existing Australian pollen data and highlighted the need for standardisation (Medek et al. 2016; Beggs

[1] The terms "fungal spore" and "spore" are used interchangeably within the document.

et al. 2015; Davies et al. 2015; Haberle et al. 2014). The best illustration of significant differences in methodology and operating procedures among pollen and spore monitoring sites across Australia is given in Haberle et al. (2014).

7.2 Scope of the Standardised Pollen and Spore Monitoring

Australia's National Health and Medical Research Council (NHMRC) funded through its Partnerships for Better Health - Partnership Projects (Partnership Projects) (NHMRC 2018, 2016) initiative the project entitled "AusPollen: Implementation of a standardized national pollen alert system for better management of allergic respiratory health". The AusPollen Partnership Project was initiated with co-sponsorship from key stakeholders including The Australasian Society of Clinical Immunology and Allergy (ASCIA), Asthma Australia, Stallergenes Australia, the Bureau of Meteorology, CSIRO and MeteoSwiss. The 10 Chief Investigators are amongst the authors and/or Reference Group for this new Standard. The AusPollen Partnership Project is building and evaluating Australia's first standardised pollen and spore monitoring network. The AusPollen Aerobiology Collaboration Network is a broader network encompassing pollen and spore monitoring sites of associated projects and extends beyond the founding sites of the AusPollen Partnership. Examples of these associated projects include the AirRater Network (led by Dr Fay Johnston; in Tasmania funded by the Tasmanian Health Department and in Canberra funded by ACT Health), the Australian Research Council Discovery Project (DP1700101630) "Satellite tracking of emerging health threats from grass pollen exposure" (Professor Alfredo Huete, University of Technology Sydney, Davies and Beggs) and the Victorian Thunderstorm Asthma Pollen Surveillance Network (VicTAPS; supported by the Victorian Department of Health and Human Services in conjunction with the Bureau of Meteorology).

This Standard and Protocols document includes requirements and recommendations for all important procedures and processes for monitoring concentrations of airborne pollen grains and fungal spores. Pollen and spore alerts and healthcare information will be delivered to patients and doctors via websites and, when appropriate, mobile applications.

8 Basis for the Standard and Protocols for Pollen and Spore Monitoring

Many sources of information have been considered in the development of this Standard (see Section 27 References). The most important information sources are: Lacey and West (2007), National Allergy Bureau (2017d), Tormo Molina et al. (2013), Rogers and Muilenberg (2001), "polleninfo.org" (polleninfo.org 2017a, b, c), Oteros et al. (2017), a document prepared by Estelle Levetin (Levetin E.), European Committee for Standardization (2015) and ANZ Standard Methods for Sampling and Analysis of Ambient Air (Standards Australia and Standards New Zealand 2016).

A primary reference for this Standard is the European Committee for Standardization's "Ambient air - Sampling and analysis of airborne pollen grains and fungal spores for allergy networks – Volumetric

Hirst method (European Committee for Standardization 2015). This Technical Specification was approved by the European Committee for Standardization on 15 September 2015 for provisional application in the following countries: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom. The period of validity of this Technical Specification is limited initially to three years. After two years the members of the European Committee for Standardization will be requested to submit their comments, particularly on the question whether the Technical Specification can be converted into a European Standard.

Moreover, the Australian/New Zealand Standard's Methods for sampling and analysis of ambient air - Guide to siting air monitoring equipment (Standards Australia and Standards New Zealand 2016) is an important, relevant, and useful reference with respect to certain features of this Standard, as is the Australian Bureau of Meteorology's "Guidelines for the Siting and Exposure of Meteorological Instruments and Observing Facilities" (Bureau of Meteorology 1997).

A number of international experts were consulted regarding the Standard and Protocols under which they operated and their awareness of other Standards and Protocols. One such expert, Dr Bernard Clot of MeteoSwiss and President of International Association for Aerobiology (IAA), provided the internal instruction files for the site and laboratory methodologies which are written in French and German. Prof Dr Jeroen Buters of the Technical University of Munich (TUM), Germany, provided a copy of the German Standard (Verein Deutscher Ingenieure 2016) which is similarly in German, as are the rules on how to set-up the sites.

9 Principles

National Principles for Environmental Information (Australian Government Environmental Information Advisory Group 2015) are listed in Table 2 and illustrated in Figure 1. These are directly and specifically relevant to this Standard.

Table 2: Principles of the Australian Government's National Principles for Environmental Information (Australian Government Environmental Information Advisory Group 2015).

Principles	Description
1. Valued	Environmental information should be treated as a valuable and strategic asset, the value of which increases through access and re-use.
2. Well described	Environmental information and data services should be described with and linked to high quality metadata that enables users to evaluate its fitness for their various purposes.
3. Standardised	Environmental information, metadata and data services should use common standards such as those identified in the National Environmental Information Infrastructure (NEII) Reference Architecture: www.neii.gov.au/publications.
4. Published online	Environmental information should be published online in a timely manner and in machine-readable standards-based formats.
5. Discoverable	Environmental information should be registered, searchable and visible through relevant web portals such as data.gov.au increasing the awareness of relevant information by government, community and industry.
6. Available under an open licence	Environmental information should be published for use under an open licensing agreement, preferably a Creative Commons Attribution (CC-BY) licence under the AusGOAL framework, and available at no cost: www.ausgoal.gov.au
7. Preserved	Data archives should be established to preserve the environmental information necessary to track changes in the environment. These archives should be aligned with the reference model for the OAIS (Open Archival Information System).
8. Governed and managed	Governance and management of environmental information collection, compilation, storage and delivery should: • implement and promote effective, efficient and consistent practices • be accountable, transparent and representative to stakeholders • support users and managers to meet these principles through education, capacity building, and facilitating the participation and collaboration of all levels of government, industry and the community.
9. Compliant	Environmental information management practices should meet statutory, legal and ethical obligations such as privacy and sensitivity.
10. Feasible and cost effective	Practices for environmental information management and publication should be feasible to implement and cost effective to sustain by organisations of all sizes, enabling the widest range of participation. This can be best achieved by aligning with the approaches of the Australian Government's National Environmental Information Infrastructure (NEII) Reference Architecture.

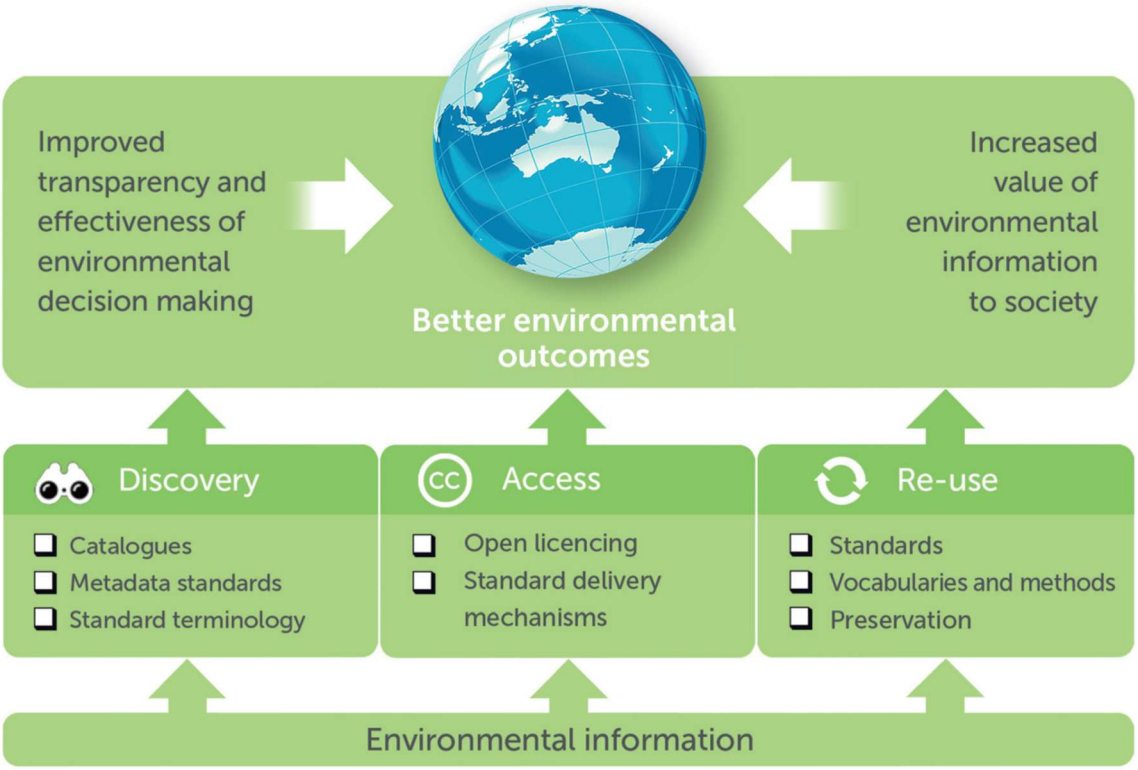


Figure 1: Increasing the value of environmental information for the environment, society and economy by improving its discovery, access and re-use (Australian Government Environmental Information Advisory Group 2015).

10 Protocols and Procedures

10.1 Guidelines on Site Selection

10.1.1 Considerations for Location of Airborne Pollen and Spore Monitoring Sites

Pollen and spore monitoring sites may serve an urban location and represent levels and timing of allergenic pollen and spore exposure for the local population. Additional peri-urban and/or rural pollen and spore monitoring sites may serve to provide information on levels and timing of release of allergenic pollen and spore sources that may be transported in the air into populated urban areas. Furthermore, there may be a need for more than one monitoring site for some locations to collect data that is representative of population exposures across a wide geographical area (Beggs et al. 2015; Katelaris et al. 2004; Rieux et al. 2008).

The site selection is one of the most important aspects of the airborne pollen and spore monitoring as the chosen location of the air volumetric sampler directly influences the results obtained. The height of a spore sampler significantly varies among the existing airborne pollen and spore monitoring sites in Australia as illustrated by Haberle et al. (2014). The literature on the influence of the spore trap height on pollen concentrations illustrates a broad spectrum of results (see Appendices, Section 26.1 Height of Volumetric Sampler). Therefore, a specific height for volumetric samplers is not defined and listed as a requirement in this Standard, but a height range is.

Co-location of air quality and meteorological monitoring sites within the vicinity of the pollen and spore monitoring sites is an option to allow for integration of meteorological and air quality data in analysis. Co-location would seem consistent with recent calls for an approach to assessment, forecasting, and communication of air quality that is integrative of the physical, chemical, and biological components (e.g., Klein et al. (2012)). Underlying this is the wealth of evidence of interactions between these components, with serious consequences for human health (e.g., Traidl-Hoffmann et al. (2009)). These interactions range from those such as the interaction between diesel exhaust particles (DEPs) and allergens through to those between thunderstorms and pollen.

The recommendations for selection of new airborne pollen and spore monitoring sites are in accordance with the requirements given in "Australian/New Zealand Standard: Methods for sampling and analysis of ambient air - Guide to siting air monitoring equipment" (Standards Australia and Standards New Zealand 2016). In particular, the specifications for monitoring of particulate matter (e.g., PM_{10}) are relevant. The recommendations for choosing the location of Pollen and Spore Monitoring sites by European Committee for Standardization (2015) and Rogers and Muilenberg (2001) were also considered (see Appendices, Section 26.1.1 Location (Including Sampler Height) Recommended by Other Standards).

10.1.2 Recommendations for Location of Airborne Pollen and Spore Monitoring Sites

Recommendations suggested by Standards Australia and Standards New Zealand (2016) and modified and recommended by Australian Airborne Pollen and Spore Monitoring Network Standard and Protocols are given below:

i. Choosing a suitable location for outdoor pollen and spore sampling to meet the requirements and aims of the project; due to the nature of the sampling equipment (Hirst style volumetric pollen and spore trap), pollen and spore monitoring is specified as "fixed point" data.
ii. Situating a volumetric sampler to collect air samples to generate data (concentrations of airborne pollen grains and fungal spores) that are representative of the location; considering that measured concentrations vary depending on sampler distance from the source and its height (vertical distance); the chosen site serves as an indicator of common pollen and spore sources for the region;
iii. Monitoring site continuity should be ensured;
iv. Avoiding the following:
    a. Sites with restricted airflows (close to buildings, trees etc.);
    b. Sites directly and excessively impacted by local extraneous biological and non-biological particle emissions
    c. Sites that may alter pollen and fungal spore concentrations or chemically and/or physically interfere with these aeroallergen sources.

v. Seeking the following:
   a. Secure sites (for personnel in accordance with WHS regulatory requirements; from vandalism and natural disasters);
   b. Easily accessible sites (for monitoring and transport of the equipment);
   c. Safe sites that can be used with low risk to personnel;
   d. Sites with adequate service (e.g., constant electricity supply).

vi. Following the minimum location criteria that includes:
   a. Height above the ground: 1.5 – 15 m;
   b. Minimum distance from supporting structures – 1m vertical and 2m horizontal (from any protruding supporting structures);
   c. The sampling inlet (orifice) should have a minimum clear sky angle of 120° (Figure 2 and Figure 3);
   d. Unrestricted airflow of 270° around the sampling inlet orifice;
   e. 10m from any object, e.g., trees (for neighbourhood & background sites);

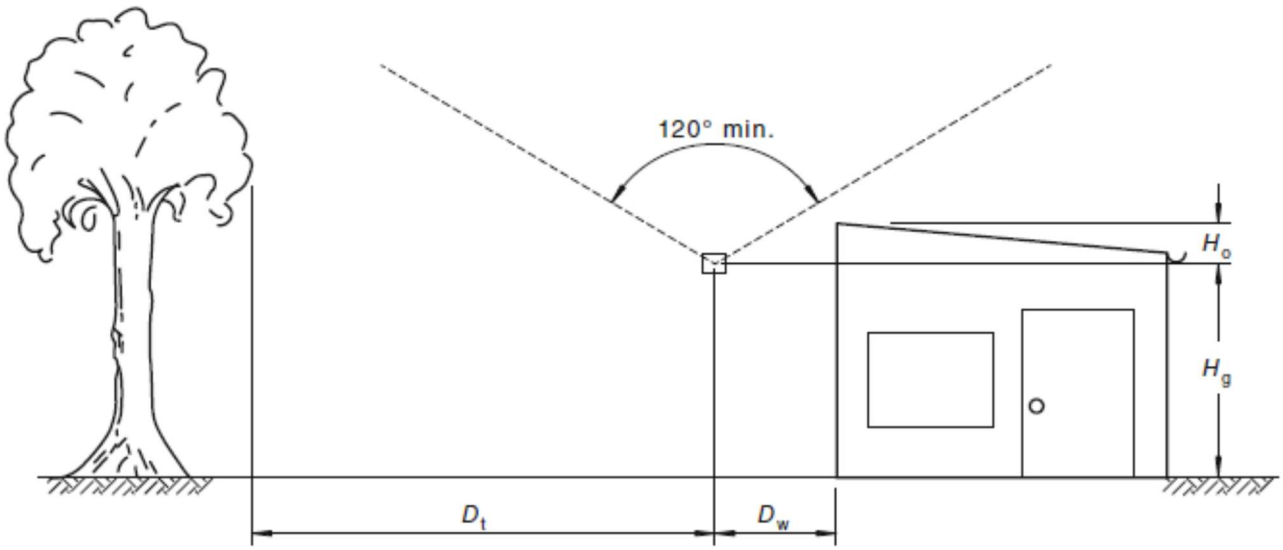


Figure 2: Generalised ground level sampling site (modified from (Standards Australia and Standards New Zealand 2016)). H_g = Height of sampling inlet (orifice) above ground—1.5-15 m; H_o = Height of nearby obstacle above sampling inlet—$2H_o \leq D_w$; D_t = Distance to nearby tree—≥10 m; D_w = Distance to wall (supporting structure)—minimum 2 m; 120° = Minimum clear sky angle above sampling inlet.

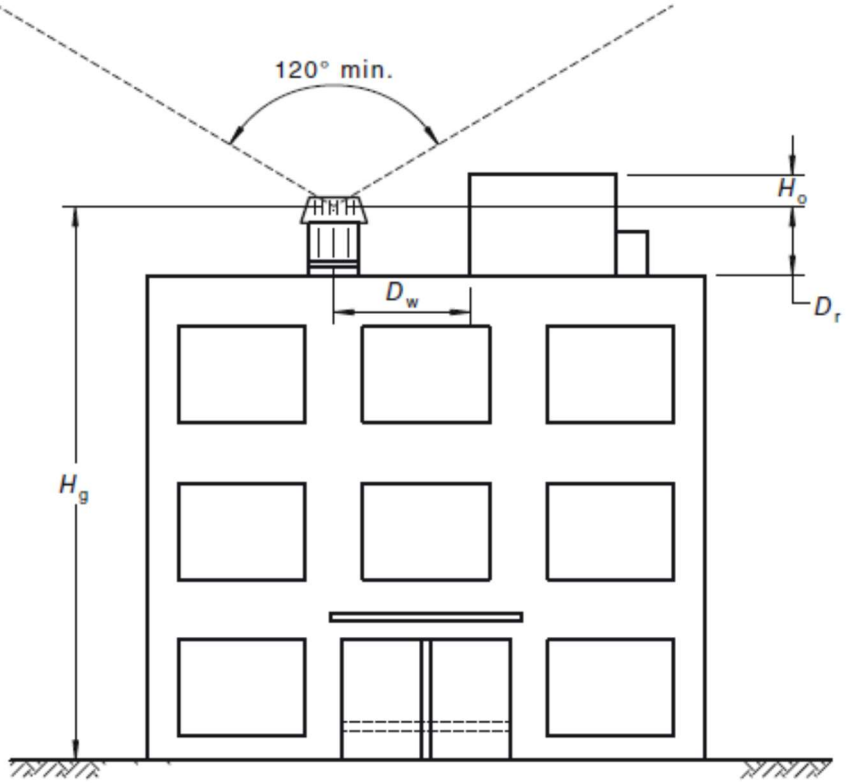


Figure 3: Generalised roof top (elevated) sampling site (modified from (Standards Australia and Standards New Zealand 2016)). D_r = Distance to roof surface (supporting structure)—minimum 1 m. Other symbols defined in Figure 2 caption.

10.2 Instrumentation and Installation

10.2.1 Instrumentation and Supporting Materials for Pollen and Spore Monitoring

All instrumentation and supporting materials for airborne pollen and spore sampling and counting are listed below.

Continuous air volumetric sampler (Hirst style volumetric pollen and spore trap) that includes the following parts:

- Built-in motorised suction vacuum pump
- 7-day drum head or 24-hour slide head
- Orifice
- Wind vane
- Rain shield
- Clockwork system
- Drum stand
- Carrying box for drum or storage box for slides

Light microscope (preferably with attached digital camera) with 10x ocular lens and 10x and 40x objective lenses

Supporting materials

Supplies:

- Microscope slides (transparent; disposable; 25 x 75 mm; 1mm thick) with frosted section for labelling
- Glass coverslips (transparent, disposable; 24 x 60 mm)
- Slide coating/particle adhesive
- Stain solution (e.g., Glycerine jelly with Fuchsin)
- Melinex tape
- Double sided and regular transparent tape
- Alcohol and cotton swabs
- Plastic pipettes
- Stain dispenser
- Laboratory tissues/wipes (e.g., Kimwipes)
- Forceps, spatula and fine scissors (or ½ razor blade)
- Connection cables (should be well insulated)

Tools:

- Spanner (for assembly and maintenance)
- Standard and Phillips head screwdrivers (for Burkard assembly and maintenance)
- Key for winding up clockwork system (supplied by manufacturer)
- Level
- Tape measure

Ancillary equipment:

- Additional drum(s) and drum stand(s)
- Additional carrying box(es)
- Flow meter
- Heating block or Microwave oven
- Cutting block
- Computer and/or calculator

10.2.2 Instrumentation installation

The following points relate to the installation of instrumentation for pollen and fungal spore monitoring.

- Assembling/setting up the sampler support platform (or roof top, etc.).
- Providing the permanent electrical support (external weather proof electricity socket with 240 Volts, 10Amp continuous power or solar panel).
- Sampler installation:
    - Secured with proper anchoring system to prevent the trap becoming loose in severe weather.

- Air quality and meteorological sites as a recommendation with tendency to be a requirement in some of the upcoming revised Australian Standard and Protocols (Figure 4).

A B

Figure 4: Example pollen and spore monitoring sites at A) Creswick, University of Melbourne, with roof mounted pollen trap on campus reception building (6.7m above ground level), and B) Richmond, New South Wales, co-located with NSW Office of Environment and Heritage Air Quality Monitoring Site.

10.2.3 Air Volumetric Sampler

The sampler designed for biological particle sampling and required by this Standard is Hirst-type volumetric sampler, Burkard. The Hirst-type volumetric sampler is a compact unit designed to collect airborne particles including pollen grains and fungal spores continuously for periods of up to seven days without attention. The airborne particles are sampled through the orifice due to the built-in vacuum pump that should ensure the regular and continuous flow rate of 10L/min (±1L/min) (European Committee for Standardization 2015). The orifice is protected from rain by a rain shield and directed towards the wind (into the air stream) using a wind vane attached to the body of the sampler which can rotate. In order to collect the particles they are impacted on an adhesive coated transparent surface supported on a continuously moving drum or a glass slide. The drum mounted with Melinex tape coated with adhesive can serve to conduct weekly or daily sampling. By using a 340mm strip of Melinex tape that covers the full surface of the drum, 7 days of continuous sampling can occur. Each 48 mm section of the transparent tape corresponds to consecutive 24 h periods, in other words the speed of the clockwork-driven drum is set on 2mm/h. Note that with a 48mm strip of Melinex tape a sample of just one 24 hour period can be collected. Alternatively, the 24-hour head assembly has a slide mounting system also driven by the same clockwork mechanism that enables a microscope slide to be moved past the orifice at 2mm/h for the 24 hour period. Use of the 24-hour head assembly for daily sampling has the advantage of collecting the daily pollen sample directly onto the surface of the

slide coated with adhesive. The volumetric sampler is made of durable, corrosion-resistant, light in weight materials and simple to operate. Details of the principle and description of the Hirst-type volumetric sampler are given in Hirst (1952). Figure 5 illustrates the most common Hirst-type 7-day recording volumetric spore samplers: those produced by Burkard Manufacturing Co. Ltd UK and Burkard Scientific Ltd UK.

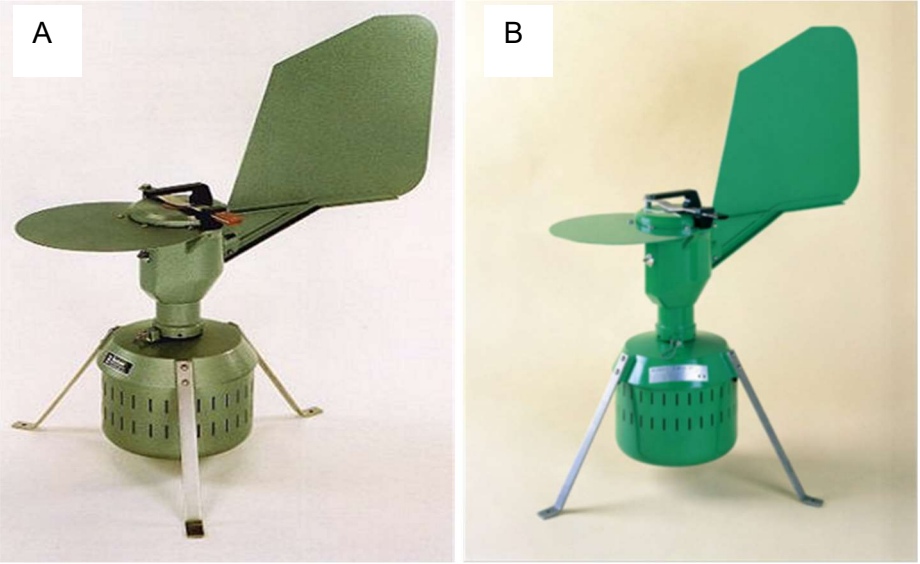


Figure 5: Example pollen and spore traps. A. Hirst (Burkard 7 Day Recording) volumetric spore sampler (right) (Image source: Burkard Scientific Ltd UK, http://www.burkardscientific.co.uk/agronomics/pdf/HirstSporeSampler.pdf) and B. Hirst (Burkard 7 Day Recording) volumetric spore trap (left) (Image source: Burkard Manufacturing Co. Ltd UK, http://www.burkard.co.uk/7dayst.htm).

10.2.4 Instrumentation Requirements and Recommendations

In order to implement the standardised methodology for all existing and newly established sites, the requirements and recommendations for instrument and supporting equipment/disposables are introduced in here.

For the pollen and spore sampling purposes the 24-hour or 7-day recording (one week without attention) Hirst-type (Hirst 1952) air volumetric sampler is required to be deployed. The commercial air volumetric samplers that meet the requirements and are recommended by the Standard are:

- The Burkard 7-day recording spore trap, by Burkard Manufacturing Co. Ltd., UK
- The Burkard 7-day recording volumetric spore sampler (240VAC (Product Code BS00285) or 12VDC/240VAC (Product Code BS02177)), by Burkard Scientific Ltd, UK
- The VPPS 2000 (or 2010) made by Lanzoni s.r.l., Italy

The volumetric sampler must be well secured with a proper anchoring system (strongly anchored to the ground or roof top) and levelled on a platform with flat, horizontal surface. The volumetric sampler must be readily- and safely-accessible by ladder, stairs, scissor lift etc. Continuous and accurate and precise sampling of pollen and spores over the sampling season (with daily or weekly harvesting) should be

performed. Furthermore, it is a requirement to maintain the sampler flow rate of 10 L/min, which is approximately equal to the volume of air inhaled by the human lung (Hirst 1952). Flow meters manufactured by Burkard Manufacturing Co. Ltd., UK are highly recommended. If a 7-day drum is used, then the transparent Melinex tape should be used. A specific type of adhesive is not required but a Silicone (polydimethylsiloxane) based adhesive is recommended.

10.3 Work Health and Safety Requirements

All work related to airborne pollen and spore monitoring in Australia must be conducted in a safe and healthy manner. Work health and safety should be a major consideration when siting a pollen trap and for all associated procedures. In this respect, those individuals involved in pollen and spore monitoring must comply with all fieldwork and laboratory rules, policies and procedures of their host institution and practise general safety awareness at all times. All relevant site-specific and organisational risk assessments, mitigation requirements and material safety data sheets for all aspects of the pollen and spore monitoring including monitor installation, monitor operation and pollen and spore slide collection/mounting/counting should be formulated, implemented and readily available before installation of a monitoring site or commencement of monitoring procedures. It remains the responsibility of site coordinator or lead investigator at each site, and their host organisation to ensure work health and safety practices are adhered to.

All personnel including staff, volunteers and students working on site (in the field) should be aware of the following general fieldwork aspects:

- Surrounding facilities (road safety, first aid kit, water, toilet etc.);
- Access policy and regulations (steps required to visit or work on the site and site rules if applicable and defined);
- Working alone policies
    - Carry mobile phone at all times;
    - Report to nominated contact person upon safe return to the working place from the field sampling within the scheduled time;
- Fire and emergency procedures;
- Medical emergency plans.

Relevant examples of common pollen and spore monitoring risks and control measures that may apply and should be considered by individual sites are provided in Table 3 and Table 4. Note that each site has the responsibility to undertake its own site-specific risk assessment and minimisation plan as well as take a responsibility to comply with their own host organisation WHS obligations. As each pollen monitoring site location is unique and there are options for methods of pollen sample collection, adhesive and slide mounting, then individual site coordinators must evaluate and manage the specific risks that apply to the specifics of thier site and pollen monitoring processes.

Table 3: Examples of pollen and spore sampling-related risks that may apply and examples of proposed control measures.

What could cause harm?	What could go wrong?	Control measures
Heat exhaustion, if outside for long periods	Heat exhaustion could occur due to sun and/or high temperature exposure and physical activity.	Wear sun protection as described below. Carry and drink water.
Ultraviolet radiation (sun burn), if outside for long periods	Sun burn could occur due to sun exposure combined with inadequate sun protection.	Wear sun protection including collared long sleeved shirt and long pants, broad brimmed hat and SPF 50+ broad spectrum sunscreen, and sun glasses. For more information visit http://www.cancer.org.au/ (preventing skin cancer) and http://www.bom.gov.au/uv/index.shtml.
Severe weather event	Rain and/or thunderstorm with the possibility of strong winds, hail and lightening.	Be familiar with local forecasted weather conditions and possible sudden changes and take decisive action before violent weather conditions occur. In case of severe weather conditions, find shelter but NOT under trees, power lines or metal structures.
Venomous snakes and spiders	To gain access to some roof-top, semi-rural or country field sites, access paths or monitoring sites may be surrounded by bush or grassland.	The site should be well maintained. Wear enclosed sturdy shoes and long pants. Watch where walking and placing hands. Personnel should be aware of how to act if they encounter a snake: https://www.ehp.qld.gov.au/wildlife/livingwith/snakes/snake_bites.html https://www.ehp.qld.gov.au/wildlife/livingwith/snakes/index.html http://www.snakecatchers.com.au/snake-information.php First aid for snake bite involves the Pressure Immobilisation Technique recommended by the relevant authority (e.g., Queensland Health or St John Ambulance (http://stjohn.org.au/). A first aid kit containing a suitable compression bandage should be carried to sites where snake bite is a risk. If you are bitten stay calm, call an ambulance, apply compression bandage, and remain horizontal. Take a photo of the snake for identification if practical to do so.
Electricity	Possible contact with electricity could cause electric shock.	The pollen and spore trap should be tested and tagged by an electrician every 12 months. The leads and electrical joint cover should be replaced annually to prevent deterioration by UV exposure. Any modification of the electrics of the Burkard during installation must be done by an electrician. Electrics should be waterproofed for outdoor use as appropriate.

Car accident	Potential car crash while travelling to perform sampling.	The driver must be licensed to drive in Australia. Private or institute vehicles must be roadworthy, registered with fully comprehensive insurance. The risk of an accident is minimised by driver experience and adherence to road rules.
Falling/tripping	Fall on the same level (including trips & slips) or fall from height when working with pollen trap on elevated surfaces (climbing ladder/ stairs and walking on an elevated surface).	Be familiar with surroundings and possible falling/tripping places. Wear fully enclosed non-slip shoes. Consider alternatives to minimise the risk of working at heights. If alternatives are not available, measures should be put in place to reduce the risk of falling such as using an appropriate and secure ladder; installing guard rails or edge protection that provides permanent fall protection; and the person should be provided with appropriate training and instruction for work at heights (e.g. Standards Australia and Standards New Zealand 2013).
General operation	Roof access, field work, high temperatures.	All staff and students who assist with the pollen and spore monitoring will be trained on proper use of the pollen and spore trap, all protocols and any other equipment. All staff and students who assist with the pollen and spore monitoring will have considered read, understood and signed the risk assessment for the pollen collection. The project supervisor is responsible for training of and oversight for all staff and students. Staff and students are responsible for their own behaviour and adherence to procedures, being aware of risks and mitigation strategies, and implementing safe work practices.
Projectile objects	The pollen trap instrument could become airborne in a storm.	The pollen trap has to be secured and strongly attached to the platform or roof surface to prevent the pollen trap from falling over or becoming airborne. Lock the revolving part of the Burkard when changing the drum or slide, conducting maintenance, or other activities near it, to avoid injury from the swinging wind vane and rain shield.

Table 4: Examples of pollen and spore mounting and counting-related risks that may apply and proposed control measures.

What could cause harm?	What could go wrong?	Control measures
The use of Lanzoni (Italy) Glycerine jelly with Fuchsin (see Safety Data Sheets in Appendices, Section 26.2 Safety Data Sheets**)**	Skin contact with hot melted solution causing Phenol burn due to not wearing gloves. Inhalation of phenol fumes by not carrying out procedure in fume hood.	The amount of phenol is low since only used in small amounts and concentration is low. If using this preparation for mounting, then personnel should consider performing the procedure in a fume hood wearing gloves, eye protection and a lab coat. In case of incident: If a burn occurs, immediately apply cold water to the burn and do not remove any clothing or gloves that have adhered to the skin. Seek medical aid. In case of a spill on skin that is not a burn, remove contaminated clothing and swab repeatedly with Glycerine. Seek medical aid. Inhalation of phenol fumes by not carrying out procedure in fume hood. If inhalation of fumes occurs remove person from area, lay them down and seek medical aid.
Cuts from broken glass slides/ glass coverslips	Sharps injury from broken glass.	Personal Protective Equipment including well-fitting lab coat, gloves and safety glasses should be used. Use care when handling. Dispose carefully into sharps containers.
Eye strain	Eye strain could develop from using the microscope for extended periods of time. Laboratory personnel could suffer from aching eyes, blurred vision, dizziness or headache.	Take periodic breaks (a minimum of 5 min every hour). Also, take a micro-break every 5 to 10 min (e.g., when completing each horizontal pass over the slide), by sitting up straight, raising head from the microscope, stretch and focus eyes on an object in the distance. Don't forget to blink since leaving your eyes open for a prolonged period dries them, and use both eyes for viewing. Personnel should not spend more than a total of 5 hours a day viewing samples under the microscope. http://safetyservices.ucdavis.edu/article/microscope-ergonomics Personnel should undertake online training in manual handling and ergonomics that is available within their organisation. Staff and students should report any issues to supervisor at an early stage so the situation can be managed before escalating to a problem.
Muscle strain (back)	Laboratory personnel could experience lower back, neck and shoulder pain during and after use of a light microscope for a prolonged period.	1. If observing samples for a prolonged period (e.g., counting multiple sites, days, weeks etc.) ensure a chair, with back rest and foot rest, is adjusted appropriately (i.e., to avoid awkward postures such as being hunched over). 2. Change between sitting and standing postures to offset static loads on muscles and change compressive forces on intervertebral discs. 3. Take a short break for a few minutes every 20-30 minutes to stretch/move around. 4. It is recommended that the one person does not spend more than 5 hours per day sitting at the microscope, however, it is desirable to minimise pollen and spore counting to two or three slides in any given day.

10.4 Sampling Time and Duration

Ideally, airborne pollen and spore monitoring should be continuous over the whole year (i.e., 365/6 days per year) and over multiple years with the aim to have continuous airborne pollen and spore sampling (Rogers and Muilenberg 2001). During the grass pollen season daily sampling, counting and reporting is highly recommended. If a 7-day sampling drum is used, then the recommended drum change days are Tuesdays, Wednesdays or Thursdays. Harvesting of a 7 day drum over the weekend is not recommended, nor Monday or Friday due to public holidays and long weekends. Sampling time for both 7-Day sampling and daily/24hr sampling is recommended to be at the same chosen time, each time between 7 and 11am. The minimum period of monitoring to encompass the grass pollen season in most states of Australia should encompass 1 October - 31 December in temperate regions (e.g., Melbourne) and 1 November – 31 March in subtropical regions (e.g., Sydney, Canberra and Brisbane).

For the Victorian thunderstorm asthma pollen forecast service, grass pollen and total concentrations need to be received at the Bureau of Meteorology in a timely manner (i.e., slide to be collected at 9 am and the pollen counts made available to the Bureau's forecaster by 11 am). Specific requirements for surveillance of grass and total pollen concentrations for this project are provided in Appendices, Section 26.3 Minimum Standards for Daily Pollen Monitoring for the Victorian Thunderstorm Asthma Pollen Surveillance (VicTAPS) Network.

10.5 Sampling Technique

The recommended techniques for pollen and spore sampling including preparation of the sample media and changing the samples for both, 7-day sampler head drum or 24-hour sampler head slide, are given in the following sections.

10.5.1 7-day Sampler Head Drum Preparation

Material needed for the preparation of the 7-day sampler head drum includes Melinex tape, double sided sticky transparent tape, adhesive, acrylic paint brush, laboratory tissues/wipes, drum stand and drum carrying box. Firstly, mount the drum on the drum stand to enable fixing of the Melinex tape and distribution of the adhesive. Clean the drum using alcohol and tissue (or alcohol swab) to remove any grease if needed. Place a piece of double sided sticky tape between the black notches on the side of the drum. Then cut the Melinex tape to the chosen length and starting from the middle black line wind it all the way around the sampling surface of the drum. Make sure that the Melinex tape is not loose. Apply adhesive on the acrylic brush and spread it evenly onto the Melinex tape. Appropriate adhesives include Sylgard or Silicone (Appendix 26.2). Note that if you are working with Silicone (polydimethylsiloxane) diluted in the organic solvents such as cyclohexane or in carbon tetrachloride, then it is highly recommended that the application of adhesive takes place using a fume hood with use of suitable personnel protective equipment (see 26.2 Safety Data Sheets). Make sure that the whole tape or slide surface is evenly daubed including edges. Wipe off any excess adhesive using the

laboratory tissues/wipes. Handle the drum using the knurled outer ring and store it in the drum carrying box before placing it onto the 7-day sampler head.

10.5.2 7-day Sampler Head Drum Changeover

Before changing the drums it is important to set up a day and a time of drum changeover. On the day of the changeover firstly make sure to fix the head with the pin in place to prevent the wind vane rotation. Measure and note the flow rate and then unlatch the head assembly and pull it out using the handle. Unscrew the nut that secures the drum and place it on a clean surface ready to secure the new drum. Carefully remove the current exposed drum and mark the tape for the end of the measurement if needed. Store the drum in drum carrying box. Label the drum carrying box with the site name, date and time of set up and date and time of harvest. Clean the orifice and exhaust port if needed. Fully wind the clock mechanism counter clockwise (once per week is sufficient) and carefully put a new drum with unexposed tape onto the drum mounting head. Make sure that the red mark on the drum is aligned with the metal arrow. Secure the drum with the nut. Carefully insert the lid assembly with the unexposed drum into the trap and close and secure the lid. Repeat the measurement of the flow rate and adjust the flow rate if needed. Remove the pin so that the head now can rotate freely. Note time and date, flow rate and any additional important information observed on the day.

10.5.3 24-hour Sampler Head Slide Preparation

Material needed for the preparation of the 24-hour sampler head slide includes microscope slide, adhesive, acrylic paint brush, laboratory tissues/wipes and slide storage box. Clean the microscope slide using alcohol and tissue (or alcohol swab) to remove any grease, dust, or dirt if needed and label the slide accordingly with the site code and date. Apply adhesive on the acrylic brush and spread it evenly onto the slide away from the label. Wipe off any excess adhesive using the laboratory tissues/wipes. Keep the slide in the storage box before placing it onto the 24-hour sampler head.

10.5.4 24-hour Sampler Head Slide Changeover

Before changing the slides it is important to set up a time of slide changeover. Once on site, firstly make sure to fix the head with the pin in place to prevent the vane rotation. Measure and note the flow rate and then unlatch the head assembly and pull it out using the handle. Carefully remove the current slide and store it in the storage box. Clean the orifice and exhaust port, and wind the clock mechanism counter clockwise if needed (once per week is sufficient). Carefully put a new slide onto the 24-hour carriage head lid assembly. Make sure the sticky side of the new slide faces the inlet and that the slide is in the start position. Carefully insert into the trap and close and secure the lid. Repeat the measurement of the flow rate and adjust the flow rate if needed. Remove the pin so that the head now can rotate freely. Note time and date, flow rate and any additional important information observed on the day.

10.6 Record Keeping (Sampling Log)

Sampling logs were obtained from some of the Australian pollen and spore monitoring groups (also see Appendices, Section 26.4 Sampling Logs from Some of the Australian Pollen and Spore Monitoring) and the recommended minimum data logging for pollen and spore monitoring is issued and implemented for the following seasons. Examples of record sheet include pollen and spore monitoring (set up and collection) record sheet (Figure 6), slide record sheet (Figure 7), and mounting and counting record sheet (Figure 8).

Pollen Monitoring Record Sheet

Burkard spore trap:
Site:
Year:

Set up date	Operator	Exchange Time	Flow rate (10L/min)	Code on drum	number of days	Exchange comment

Figure 6: An example of pollen and spore monitoring (set up and collection) record sheet.

Pollen and Spore Slide (year or season or date period)

Site Number (or name or code)
Air Volumetric sampler number (or code or unit)

Sampling date and time (date and time of first day of sampling if drum is used)	Code on slide	Slide number (if drum is used)	End time day 7 (if drum is used)	Sampler Operator (Full Name or Initials)	Operator/ mounted by (Full Name or Initials)	Date slide mounted	Comments

Figure 7: An example of pollen and spore slide record sheet (7-day Sampling Head).

Pollen Counting (year or season or date period)					
Site number (or name or code):					
Air volumetric sampler number (or code or unit):					
Operator/counted by (full name or initials):					
Sampling date:					
Mount date:					
Count date:					
Microscope and magnification used:					
Representative photos taken (image file number):					
Mount and count comments (any issues with the slide, e.g., high amount of debris):					
Raw counts					
Taxa	Longitudinal Transect				Daily Total Count
	1	2	3	4	
Arecaceae					
Asteraceae					
Betulaceae					
Casuarinaceae					
Chenopodiaceae					
Cupressaceae					
Cyperaceae					
Fabaceae					
Myrtaceae					
Oleaceae					
Pinaceae					
Plantaginaceae					
Platanaceae					
Poaceae					
Ulmaceae					
Urticaceae					
Other (unknown/unspecified)					
Broken pollen (optional: must be					

identifiable as pollen by size, structure and staining)					
Fungi:					
Davidiellaceae					
Didymellaceae					
Pleosporaceae					
Trichocomaceae					

Figure 8: An example of pollen mounting and counting record sheet.

10.7 Mounting and Count Method

10.7.1 Preparing and Mounting the Slides (7-day Sampling Head)

Pollen on Melinex tape or slides must be mounted for visualisation by light microscopy. Pollen can be stained with fuchsin to aid identification. Calberla's stain is one mounting media recommended for pollen staining. This stain can be made following the recipe given in the Appendices, Section 26.5 Preparing Calberla's stain (The University of Melbourne Undated). Use of Calberla's solution has the advantage that it is low cost and does not require heating or fumehood for use. If using Calberla's stain solution, then to prevent dehydration of the slide that affects visualisation of the collected pollen, the coverslip edges must be sealed to the slide with sealant or nail polish. Alternatively, Glycerine jelly with Fuchsin (available from Lanzoni s.r.l., Italy) can be purchased. Glycerine jelly with Fuchsin does not evaporate thus slides do not need to be sealed for storage and counted at a later date if needed. However, the slides should be mounted using a fumehood because the jelly contains traces of phenol, and it must be gently heated before use (see Appendices, Section 26.2 Safety Data Sheets). The following paragraphs include recommended techniques for slide preparation and slide mounting.

If using Glycerine jelly with Fuchsin, it is highly recommended that preparation and mounting of the slides be carried out in a fume hood and using personal protective equipment (lab coat, gloves and safety glasses). Place a heat block into the fume hood and set to 50-55°C. Melt the Glycerine Jelly with Fuchsin; scoop a small amount (2-5 ml) of Glycerine Jelly with Fuchsin into a polypropylene vial or glass jar with a metal spatula and place on the heat block. Clean the spatula with ethanol and a lint free tissue. Label the slides accordingly with site code and date and place onto the heat block. Using a disposable 1ml pipette, place 3 small drops of the Glycerine jelly with Fuchsin evenly onto the slide.

Once everything is ready for the slide mounting, place the pollen drum on the stand into the fume hood. In order to remove the exposed Melinex tape from the drum, hold the drum in the left hand by the slots and lift a corner of the tape with forceps (with the help of spatula) and then peel off the strip. The green line on the drum indicates the commencement of the sampling period, the black line the

completion. If you pull the tape downwards towards you, that will be the last day of sampling. Roll the tape onto the cutting block and secure the end of the Melinex tape with the double sided sticky tape and position the Melinex tape to the start mark. Each segment should then be cut using the fine scissors into 24h sections by using the cutting block marks (48mm - 24h). Lift the tape by sliding the scissors under the tape and holding it with the forceps. Once tape is cut, apply it gently to the slide starting from one end and placing it along the slide and if you see bubbles lift the tape and place it again or press the tape with forceps to release the bubbles. Apply another 3 small drops of glycerine jelly with fuchsin along the top of the tape. Cover the slide with glass coverslip using thumbs and forefingers of both hands. If needed, remove bubbles and any excess stain using spatula or tissues on the slide edges. Take the slide off the heat block and cool for a few minutes at room temperature in the fume hood to evaporate any traces of phenol from the gelatine stain before the microscopy examination. Ensure that once mounting is finished, the working area is properly cleaned with ethanol.

10.7.2 Preparing the Slides (24-hour Sampler Head)

Firstly, prepare the mounting solution (see Appendices, Section 26.5 Preparing Calberla's stain; The University of Melbourne, Undated) or Calberla's solution. Place 3 small drops of the Glycerine jelly evenly onto the sticky side of the slide. Cover the slide with glass coverslip using thumbs and forefingers. If needed, remove bubbles and any excess stain using cotton swabs or spatula or tissues on the slide edges. Take the slide off the heat block and cool for a few minutes at room temperature in the fume hood to evaporate any traces of phenol from the gelatine stain before the microscopy examination. Ensure that once mounting is finished, the working area is properly cleaned with ethanol.

10.7.3 Pollen and Spore Identification and Counting

The identification and counting of pollen grains and fungal spores can be performed with any light microscope that ensures a high quality and resolution image. An example of an appropriate light microscope is the Nikon Eclipse E-200 microscope. The required microscope magnification is 400x (40x objective and 10x ocular lens).

The minimum examined surface of the slide should be 10%, as recommended by Mandrioli et al. (1998) and Galán et al. (2014) and supported by the study of Šikoparija et al. (2011). Galán et al. (2014) observed that more of the counters, who did not follow the minimum recommendations, were found to be outside of the thresholds of relative errors compared to counters who did follow the minimum recommendations (including 10% of the slide). The recommendation that 10% of the slide should be counted was included in the updated Minimum Requirements suggested by the European Aerobiology Society (EAS) Quality Control Working Group (European Committee for Standardization 2015). The required counting method is the longitudinal (horizontal) transect method (European Committee for Standardization 2015). As it would be time-consuming to count all pollen grains and fungal spores on the whole slide, several longitudinal transects (minimum 3; 4 recommended) should be selected to provide coverage of the average daily concentrations. Notably, longitudinal transects

provide for capturing diurnal variations in release of pollen and airborne transport of pollen. It is recommended to select four longitudinal transects that are equal distances from each other and the edges of the tape/slide (e.g., on the 14 mm wide Melinex tape transects should be centred at 2.8 mm, 5.6 mm, 8.4 mm and 11.2 mm from the side of the tape).

10.8 Calculations

The average sampling time for data reporting is to be daily (Standards Australia and Standards New Zealand 2016). Atmospheric pollen or fungal spore concentrations should be expressed as the daily average pollen grains or fungal spores per cubic meter of air. This can be calculated using the following equation, which takes into account all the relevant factors (Rogers and Muilenberg 2001; European Committee for Standardization 2015).

$$[\text{Pollen or Spore}] = n\left[\frac{L \times W}{L \times (\frac{F}{M}) \times N} \times \frac{1}{D \div 1000 \times t}\right]$$

where

[Pollen or Spore] - atmospheric pollen grain or fungal spore concentration per cubic metre

n - number of pollen grains or fungal spores counted in the analysed area of the microscope slide

L - length of impaction area on the melinex tape or glass slide (mm) e.g., 48 mm

W - width of impaction area on the melinex tape or glass slide (mm) e.g., 14 mm

F - field number written on the eyepiece (typically a number between 18 mm and 25 mm)

M - objective magnification (typically 40)

N - number of transects e.g. 4

D - flow rate of the equipment (l/min) e.g., 10 l/min

t - duration of the sampling period (min) e.g., 1440 min

These formulas can be programed into a Microsoft Excel spreadsheet that can be used by all Australian pollen and spore monitoring sites to ensure consistent use of the same formula and the correct formula (adjusted for any differences in field of view of the microscope or number of transects counted).

The diameter of the microscope field of view is calculated based on field number of diameter of the image observed through the eyepiece (e.g. 18-22 mm) divided by the objective lens magnification e.g. 40 x. For example the field of view for a microscope with an ocular diameter of 18 mm when using a 40 x objective magnification is 18/40 = 0.45 mm (Table 5).

Table 5 Field of View diameters (d) for common light microscope eyepiece field number diameters.

Eyepeice diameter of view (field number)	Field of view diameter (d) with 40x objective lens
18 mm	0.45 mm
20 mm	0.50 mm
22 mm	0.55 mm

10.9 Maintenance

The following bullet points indicate recommendations for maintenance of the volumetric sampler and microscope:

- Power supply: Electrical safety testing and servicing of the volumetric pollen and spore trap and other site-specific equipment are to be done annually. Continuous power supply should be ensured. The power cord and connection cover (between the pollen trap and the power lead) should be checked every 12 months and replaced as necessary to minimise risks associated with deterioration due to outdoor conditions.
- Air flow: Checking the flow of all traps used in Australia with a standard NATA accredited flowmeter annually for calibration is recommended.
- Pollen and Spore Trap: All bolts and nuts have to be regularly checked and replaced if needed. Air volumetric sampler should be properly and regularly cleaned, as well as lubricated by applying a high vacuum Silicone grease to surfaces of sample heads and locking bar latch (National Allergy Bureau 2017d). The rotation of the clock work mechanism should also be checked annually and adjusted as necessary.
- Microscope: Proper and regular cleaning of microscope surfaces and lenses should be ensured.

11 Training and Certification

11.1 Training and Certification of Counting to Minimum Required Standard

Well-established and high quality training programs exist in Europe (European Aerobiology Society 2017) and North America (National Allergy Bureau 2017c). The International Association for Aerobiology and European Aerobiology Society run two courses: the European Course on Basic Aerobiology and the European Course on Advanced Aerobiology (European Aerobiology Society 2017; International Association for Aerobiology 2015b, a). In the US, counters are certified separately as a pollen counter or as a spore counter (National Allergy Bureau 2017b, c). Following these models, pollen and spore counter training and certification will be established in Australia through the AusPollen Aerobiology Collaboration Network in conjunction with the Australasian Aerobiology Association (AAA) and ASCIA. A joint Working Group for the AusPollen Aerobiology Standard is being established through AAA and ASCIA and will be comprised of membership by AusPollen Aerobiology Collaboration

Network participants who are members of both or either AAA or ASCIA. Terms of Reference and Lines of Reporting for this joint Working Group will be provided as an appendix to this document (late 2018/ early 2019). The Working Group for the AusPollen Aerobiology Standard will be responsible for

i) setting criteria for compliance with this Standard and Protocols document,
ii) ii) certifying registration of Australian Pollen and Spore Monitoring sites,
iii) iii) training and certification of individuals registered for counting pollen and spores,
iv) iv) auditing of sites, and
v) v) periodic review of this Standard and Protocols document.

The pollen and spore monitoring training will be developed through a multi-modal approach including training workshops and online e-training modules. The AusPollen Aerobiology Working Group will develop this training program which will be provided as an appendix to this document in 2018.

11.2 Certification of Counters

The pollen (and spore) counter certification process will include training and pollen and spore counting exercises organised for all personnel working in pollen and spore monitoring to become certificated counters. The candidate applying for certification will be required to take an online e-training module and qualifying exam as well as a practical exam identifying and counting local relevant Australian pollen or fungal spores on a single slide. Individuals can choose to apply for “basic” accreditation that includes an online theory test and practical exam of skills in grass and total pollen identification and counting, and “advance” accreditation that includes practical exam with all or specified pollen and spore taxa (that are relevant to local Australian environment) identification and counting. Personnel will be required to renew their certification every 5 years (before the beginning of the season) by redoing the web-based qualifying theory exam, participating in quality control and auditing activities (Section 13 Quality Control and Auditing), or redoing the certification practical exam. Personnel will be also highly advised to attend national training workshops in pollen and spore monitoring through the AusPollen Aerobiology Collaboration Network and/or international courses with a particular emphasis on: identification of the common Australian and exotic allergenic pollen types, how to manage the pollen sampler and preparation of the sampling slide.

11.3 Certification of Sites and Operators

The Australian Standard and Protocols recommends all registered monitoring sites and all site operators and counters to be certified. The certification process will involve an application to register a pollen and spore monitoring site with the AusPollen Aerobiology Collaboration Network. Once a pollen and spore monitoring site has been certified and registered with the AusPollen Aerobiology Collaboration Network, it will be assigned a site name and number according to an agreed convention (in preparation). Certified sites will be asked to provide information about their site to be included in the National Environmental Monitoring Sites Register (see Appendices, Section 26.6 Summary of

Metadata Fields for the NEMSR) to manage and store pollen and spore data according to the data management plan (Section 18 Data Archive and Repository).

12 The Australian Aeroallergen Monitoring (AusPollen) Network

Pollen and spore monitoring and related activities and services at the national (and regional) scale are best achieved through a coordinated, standardised, and systematised network. Advanced examples of such networks include the European Aeroallergen Network (European Aeroallergen Network 2015), the UK National Pollen Monitoring Network (University of Worcester 2017; Met Office 2017) and the AAAAI's Aeroallergen Network (National Allergy Bureau 2017a). The AusPollen Aerobiology Collaboration Network provides a service and a database for the pollen and spore data that aims to provide allergy and asthma patients with accurate, relevant, localised information on pollen concentration. Information on airborne pollen and spores generated by sites registered with the AusPollen Aerobiology Collaboration Network will lead to reduced symptoms, improved quality of life, and will empower patients to self-manage their condition.

12.1 Registration Requirements

Sites seeking registration as part of the Australian Aeroallergen Monitoring Network must provide the metadata described in Table 11 and Table 12. These tables are part of the document that provides a summary of metadata fields and a worked example for the National Environmental Monitoring Sites Register (NEMSR). The full version of the NEMSR information model, including vocabularies and validation rules, can be found at: www.neii.gov.au/nemsr/documentation.

12.2 Maintaining registration

Maintenance of site registration requires reasonable compliance with this Standard, appropriate accuracy of pollen and fungal spore identification, quantification and subsequent reporting. Maintenance of registration will also require participation in and satisfactory outcomes of quality assurance exercises at least once every three years. Sites whose pollen counters are not certified or that do not meet these requirements and, following identification of this, that have been given the opportunity to rectify the situation but have not responded within a reasonable time period (one year), may have registration reviewed by the Working Group.

13 Quality Control and Auditing

Quality control of pollen and spore monitoring of counter reliability will be performed including the internal and external validation of counted samples. The internal validation will be organised among staff members within same working groups using quality control exercises that include validation of counting on practice slides. External validation of counted samples will encompass the quality control exercise examining counting proficiency of certified counters at participating sites of circulated reference slides. The quality assurance exercises will be facilitated by the AusPollen Aerobiology

Collaboration Network. The pollen counter performance characteristics will be estimated considering precision (repeatability and reproducibility) and accuracy (Oteros et al. 2013; Galán et al. 2014; European Committee for Standardization 2015).

The quality assessment process has been designed in accordance to published approaches applied by other network standardisation studies (Oteros et al. 2013; Galán et al. 2014) (see Appendices, Section 26.7 Other Network Quality Assurance and Standardisation Studies). The following bullet points illustrate the Quality Assessment steps:

- Count the grass and total pollen using as a guide a local pollen image library supplied by the slide submitter and developed by the AusPollen Aerobiology Collaboration Network.
- Slides may be selected from three times of year/season with different dominant species. Selected slides should have between 100 and 250 grains per cubic meter total pollen concentration.
- Each counter at each site counts and submits counts for the set of 2 slides.
- Original reported values for the reference slides that have had the total area counted to give the total daily concentration that will be used in comparison as the true value.
- Analysis of variation of the individual pollen counters for each slide will be determined against the average true value for three experienced counters (Galán et al. 2014)

13.1 Site Audits

Whilst not essential for site registration, a site audit may be conducted by members of the AusPollen Aerobiology Collaboration Network Working Group to evaluate the characteristics of new and existing pollen and spore monitoring sites, check instrumentation set up conditions, the flow rate of the pollen and spore trap, data logging and overall compliance with practices described in this Standard and Protocols. An external site audit may be requested by the site operator for their own benefit or by funding bodies or other authorised organisations. The site to be audited must agree to be audited before a site audit will proceed. The site will then provide access to the site and cooperate in the auditing process. A site audit report will be provided to the site operator and to the organisation that requested the external audit. The audit report may include recommendations for modification to the setup of the site or to the monitoring and data management processes. The site operator will take reasonable steps to implement the recommended changes and report to the Working Group within 2 months of receiving the report. If reasonable efforts have not been taken to address the recommended changes, then the Working Group may opt to review the registration of the site.

The audit checklist should be used to consistently evaluate the set-up of pollen monitoring sites and their overall compliance of processes for monitoring airborne pollen including collection, processing, counting and reporting of the airborne pollen concentrations based on this Australian Airborne Pollen and Spore Monitoring Network Interim Standard and Protocols. The site audit checklist was designed

by QUT to evaluate compliance with ten key criteria specified in the minimum standards and recommendations of this Standard and Protocols (See Appendix 26.9 Pollen Monitoring Site Audit Checklist).

14 Pollen and Spore Lists

All the pollen and fungal spore families recommended to be monitored and reported by sites registered in the AusPollen Aerobiology Collaboration Network are listed in Table 10 and Table 11. All sites should include the taxa that are abundant sources of pollen in the region and that represent the local composition of airborne pollen and spores. Fungal spore allergens are observed to be an important factor in hospital asthma admissions (Davies et al. 2017), therefore monitoring of certain fungal spore taxa (Table 7) may be informative of risks of exposure and is recommended. It is recommended to record all taxa observed including other/unknown (unspecified) taxa that can be counted. Counting should include pollen grains on the edge of the field of view. Ruptured or broken pollen grains may be counted as a separate entry. Preferably reasonable efforts to discover the botanical family of unidentified pollen should be undertaken through review of relevant literature and consultation with experts to identify unknown pollen types (e.g. consult the Australasian Pollen and Spore Atlas: http://apsa.anu.edu.au/). The minimum taxa required to be monitored and reported by sites registered in the AusPollen Aerobiology Collaboration Network are grass and total pollen. The taxa that each site does count can be included in site registration metadata files so availability of data for particular locations on concentrations of other types of airborne pollen and spores is known.

14.1 Scientific and Common Names

The plant and fungal families listed in Tables 5 and 6 are based on research of the most common plant pollen and fungal spore allergies in Australia (Wjst et al. 2005; Bousquet et al. 2007; Kam et al. 2016); as well as particularly important invasive/sleeper species (e.g., ragweed) (Beaumont and Duursma 2016) (Table 6 and Table 7). These tables will be expanded/contracted following further review of literature and expert input.

Table 6: Pollen families recommended to be monitored and reported by sites registered in the AusPollen Aerobiology Collaboration Network. Exemplary allergenic species within each family and common names are also shown.

Family name	Genus name (example)	Species name (example)	Common name
Arecaceae			
Asteraceae	*Ambrosia*	*artemisiifolia*	Ragweed
Betulaceae	*Betula*	*pendula*	Birch
Casuarinaceae	*Allocasuarina*		She oak
Chenopodiaceae			
Cupressaceae			
Cyperaceae			
Fabaceae			
Myrtaceae			
Oleaceae	*Olea*	*europaea*	Olive
Pinaceae	*Pinus*	*radiata*	Pine
Plantaginaceae	*Plantago*	*lanceolata*	Plantain
Platanaceae	*Platanus*	*acerifolia*	London plane, hybrid plane
Poaceae	*Lolium* *Phleum* *Cynodon* *Paspalum* *Sorghum*	*perenne* *pratense* *dactylon* *notatum* *halepense*	Perennial ryegrass Timothy grass Bermuda grass Bahia grass Johnson grass
Ulmaceae			
Urticaceae	*Parietaria*	*judaica*	Parietaria

Table 7: Fungal spore families recommended to be monitored and reported by sites registered in the AusPollen Aerobiology Collaboration Network. Significant species and common names are also shown.

Family name	Genus name	Species name	Common name
Davidiellaceae	*Cladosporium*		
Didymellaceae	Didymella		
Pleosporaceae	*Alternaria*	*alternata*	
Trichocomaceae	*Aspergillus* *Penicillium*	*fumigatus*	

15 Pollen and Spore Image Reference Library

Registered sites and pollen counters will be invited to submit images of pollen grains observed in their location to generate an Australian pollen and spore image library of pollen grains and fungal spores visualised by light microscopy and stained with Fuchsin. The pollen grain and fungal spore image library will be developed and managed by members of the AusPollen Aerobiology Collaboration Network in a way that is similar to or incorporated within the Australasian Pollen and Spore Atlas (ANU), the Atlas of Living Australia or the Australian Virtual Herbarium and Pollen Atlas: Pollenwarndienst. The process for development and uploading images of the AusPollen Aerobiology Collaboration Network Image Library will be provided as an appendix to this document in 2018.

16 New Technologies

Adoption of new technologies that improve pollen and spore monitoring is encouraged. Such technologies include automated pollen counters, which may increase the quantity and/or quality of aeroallergen information derived from monitoring, and decrease the time and cost associated with obtaining such information. Until sufficient evidence exists that new technologies are suitable and sustainable improvements to the protocols detailed in this Australian Aeroallergen Standard, they should be used in parallel with the Standard and Protocols detailed herein.

A number of automated real-time pollen monitor prototypes, which are based on a variety of technologies, are under development (Oteros et al. 2015; O'Connor et al. 2014). There are no systems currently in production commercially and the available prototypes are expensive, have low accuracy at low pollen concentrations and have not been tested in Australian environmental conditions. However, if such devices were evaluated and appropriately adapted to Australian conditions, then these could in future complement or substitute for existing pollen and spore monitoring sites and/or equipment.

Automated pollen and spore monitoring instruments should be tested for their utility and accuracy in Australia under local conditions for relevant types of exposure levels and composition. The devices would need to be robust for the Australian environmental conditions, produced by a company with a sustainable business model capable of providing long-term technical support and software maintenance. Automated pollen and spore monitoring capabilities would need to be sensitive and specific for airborne grass and other pollen at concentrations that occur in Australia. Alternatively, prototype devices could be developed in collaboration with existing experts from overseas companies or independently in Australia.

However, such new technologies will likely also have some costs and disadvantages. Both the advantages and disadvantages as well as benefits and costs should be considered before adopting new technologies. For example, Bastl et al. (2017) detail the pitfalls for pollen information by means of automated pollen counting.

The pollen concentration data from automated pollen and spore monitoring and/or current methods could be integrated with greenness indices derived from remote sensing to provide a wider ground-truthing of grass pollen levels across Australia and contribute to a broader pollen aerobiology data network (Restrepo-Coupe et al. 2016; Devadas et al. 2018).

Pollen concentrations as a measurement alone are not necessarily representative of exposure to the allergen component within the pollen grain which can vary between cultivars, with environmental conditions, plant maturity and over time during the season (Davies et al. 2015). It has been suggested that monitoring the allergen content together with pollen and spore concentrations in ambient air might improve assessment of allergen exposure (Buters et al. 2012; Galán et al. 2013). Therefore the analysis by immunoassay (Schäppi et al. 1999) or protein analysis (e.g., two dimensional gel electrophoresis) (Buters et al. 2015) of aeroallergen content using high volume air sampler (e.g., ChemVol High Volume Cascade Impactor; High Volume Total Suspended Particulate Air Sampler) instrumentation located at the same site as the spore trap may be informative and beneficial and it is therefore recommended if there is capacity to do so.

17 Existing Pollen and Spore Monitoring Sites in Australia

Information about existing pollen and spore monitoring sites in Australia can be found on NEMSR (http://www.neii.gov.au/nemsr) under the AusPollen Aerobiology Collaboration Monitoring Network title, including the founding sites of the AusPollen Partnership (also available at (AusPollen)) the AirRater sites (also available at https://airrater.org/); the VicTAPS sites; and new sites associated with an ARC Discovery Project. This list will be updated to include additional AusPollen Aerobiology Collaboration Network sites when the information from associated project sites becomes available through implementation of the processes for site registration (including site metadata). The AusPollen Aerobiology Collaboration Network sites are visible on website http://neii.gov.au/viewer/ by clicking “add data” and choosing under the category of “Air” the “AusPollen Aerobiology Collaboration Network”).

18 Data Archive and Repository

18.1 Daily Grass and Total Pollen Concentration Data

Stewardship of digital environmental data has received increasing focus and attention in recent years in recognition of its importance (e.g., Peng et al. (2016a) and Peng et al. (2016b); Figure 10 and Figure 11. The AusPollen Aerobiology Collaboration Network has a responsibility to establish and maintain appropriate data stewardship of both past and future Australian pollen and fungal spore data. It is under consideration that daily data feeds of grass pollen and total pollen concentrations will be captured in real time and stored securely in directories with restricted access on the National Computational Infrastructure (NCI) facility (Figure 9). This facility will provide a backup system for our

daily data feeds, which is independent and not a university site. It will also provide a good integration of our data with other datasets. The hosting infrastructure will be provided by NCI through the 10 PB RDS national data collection. This collection maintains datasets of national significance from the fields of astronomy, ecology, geology, meteorology, climatology, and oceanography. Data access is provided using THREDDS using the ISO 19115 geospatial metadata schema. The Australian Airborne Pollen Data Repository Proposal is given in Appendices, Section 26.8 Australian Airborne Pollen Data Repository Proposal (for the National Computational Infrastructure repository) (August 2017).

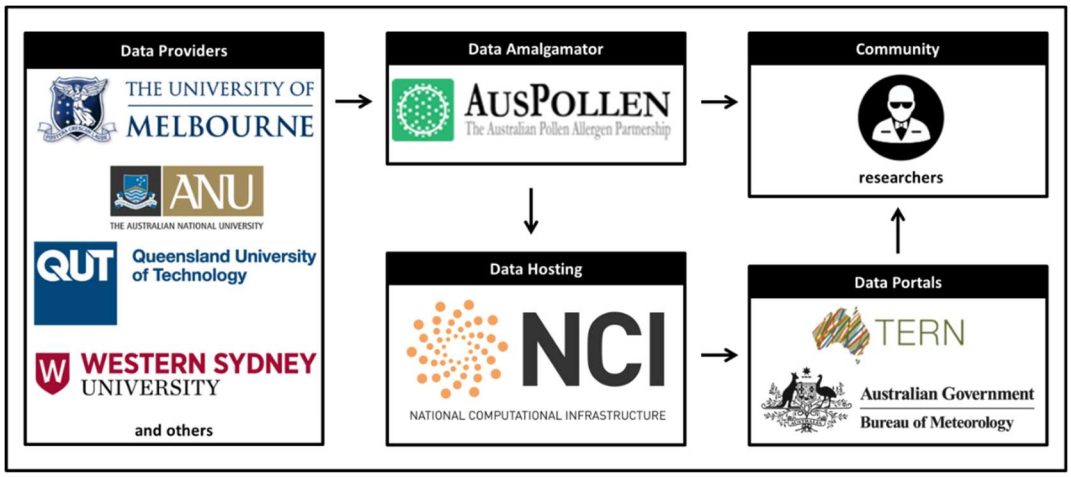


Figure 9: Overview of National Computational Infrastructure data hosting, portals and community workflow for daily grass and total pollen concentration data. (*Figure prepared by Dr Edwin Lampugnani, The University of Melbourne*).

18.2 Metadata for the Airborne Pollen and Spore Monitoring Sites

In partnership with the Bureau of Meteorology, metadata about the pollen and spore monitoring sites is included in the National Environmental Monitoring Sites Register (NEMSR) which is a widely accessible portal of Australia's environmental monitoring sites (Table 10, Table 11 and Table 12, Section 26.6).

18.3 Archive of the Total Season Pollen and Spore Datasets

It is under consideration that each year at the end of each season an archive of airborne pollen and spore data for each taxa that has been counted at a particular site will be checked and converted to a 7 day sum of daily concentrations. This annual curated data set will be provided by the site owner and archived on the data portal of the Terrestrial Ecosystem Research Network site as a publicly accessible dataset for additional research use. The data will be provided on this site under a creative commons share alike non-commercial attribution license version 3.

Maturity Scale / Key Component	Level 1 - Ad Hoc Not Managed	Level 2 - Minimal Managed Limited	Level 3 - Intermediate Managed Defined, Partially Implemented	Level 4 - Advanced Managed Well-Defined, Fully Implemented	Level 5 - Optimal Level 4 + Measured , Controlled , Audit
Preservability	*The state of being preservable*				
Accessibility	*The state of being publicly searchable and accessible*				
Usability	*The state of data product being easy to understand and use*				
Production Sustainability	*The state of data production being sustainable and extendable*				
Data Quality Assurance	*The state of data product quality being assured/screened*				
Data Quality Control / Monitoring	*The state of data product quality being controlled and monitored*				
Data Quality Assessment	*The state of data product quality being assessed*				
Transparency / Traceability	*The state of being transparent, trackable, and traceable*				
Data Integrity	*The state of data integrity being verifiable*				

Figure 10: Conceptual diagram showing the nine Data Stewardship Maturity Matrix (DSMM) key components, 5-level scale structure, and high-level descriptions of what each key component measures (Source: Peng et al. (2016a)).

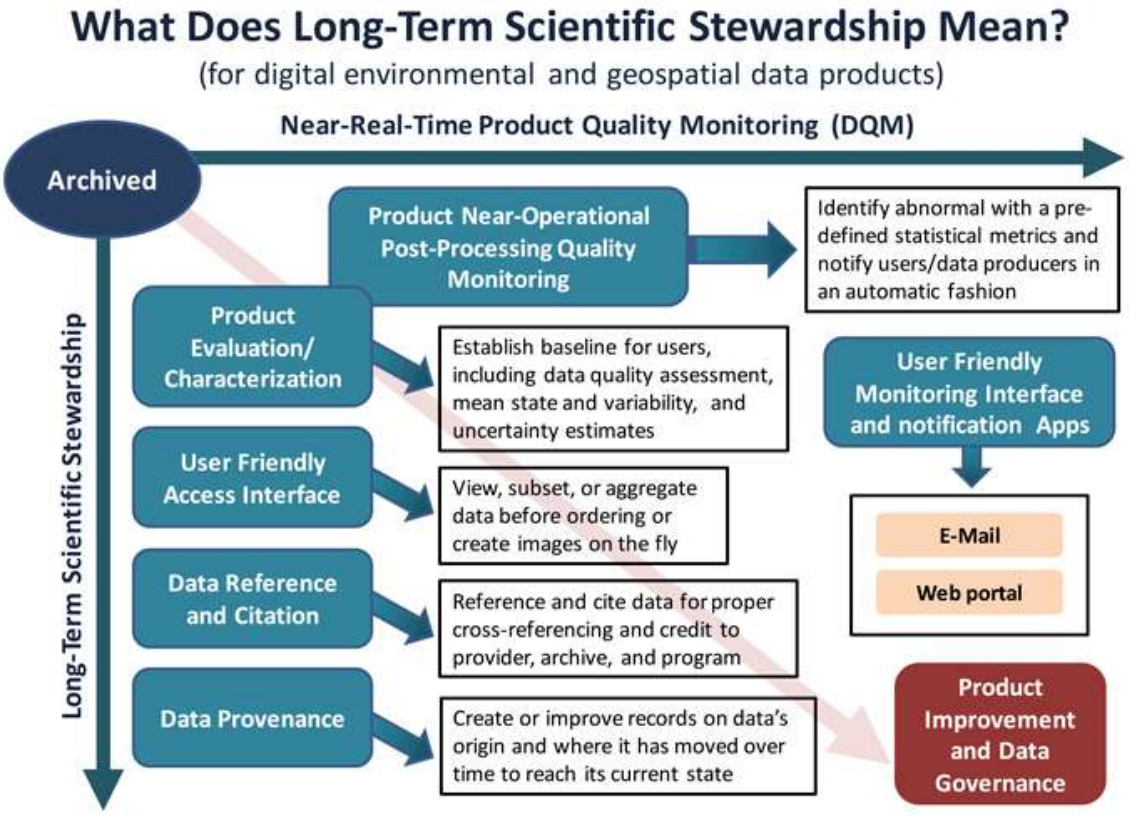


Figure 11: Diagram of functional areas (cyan-filed boxes) for scientific stewardship of digital environmental data products (Source: Peng et al. (2016b)).

19 Data Ownership and Licensing

Daily pollen and fungal spore data are owned by the respective site operators and their institutions. A Creative Commons (CC) 3.0 Restrictive License Agreement will be applied to the pollen and spore concentration datasets. Arrangements for the sharing, release, and use of aeroallergen data in other countries/regions, and other environmental data in Australia, can be considered in the development of such arrangements for aeroallergen data in Australia (see Appendices 26.6 and 26.8). Examples of license arrangements for sharing of pollen and spore data include the NAB Data Release Guidelines (National Allergy Bureau 2013).

20 Definition of Pollen Season, Peak Pollen Period, High Pollen Days

Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis (Pfaar et al. 2017).

To date, no standardised approach has been adopted for determining the pollen season in Australia. Methods of determining the start and the end of the pollen season required by this Standard are the 90 and 98% methods (Andersen 1991; Galán et al. 1995b; Jato et al. 2006). According to the 90% method the pollen season is defined as the period starting when the sum of daily mean pollen concentrations reaches 5% of the annual total sum and ending when the sum reaches 95% of the annual total sum, i.e., the interval with 90% of the annual pollen amount. According to the 98% method the pollen season is defined as the period starting when the sum of daily mean pollen concentrations reaches 1% of the annual total sum and ending when the sum reaches 99% of the annual total sum, i.e., the interval with 98% of the annual pollen amount.

21 Public Reporting

Methods for public reporting of data are varied and under ongoing development. Examples include direct distribution to the community of pollen concentration information daily via the smart phone applications available online (www.pollenforecast.com.au) from sites including Melbournepollen.com.au (which also includes regional Victoria), Brisbanepollen.com.au, Sydneypollen.com.au and Canberrapollen.com.au as well as University of Tasmania AirRater https://airrater.org/. The dissemination plan and methods for uploading pollen concentration data for providing pollen information to the public and health services are under development and will be provided in detail in future versions of this Standard and Protocols.

22 Public Forecasting

Forecast models have been developed to predict local pollen aerobiology and assist in management of symptoms and disease and reduce the health and socio-economic burden of pollen-induced allergies. Pollen forecasting methods tend to be empirically-derived composites of expert knowledge, weather

and in some cases, patients' symptom reports (personal communication; Thibaudon M. 2015. We don't use model for the forecast of the days after, we use a manual equation). Meteorological information (temperature, relative humidity and precipitation) is typically regressed with atmospheric pollen concentration data, however, these site-based empirical relationships are hampered by a sparsity of sampling sites and are generally not amenable to cross-site generalisations and latitudinal comparisons. Notably, pollen forecast methods for temperate areas cannot be generalised to subtropical sites (Green et al. 2004). The meteorological information is used to predict plant growing season phenology and estimate flowering times from growing degree days. It is important to take into account the pollen release from plants (emissions) from near- but also possible far-sources of the pollen and pollen characterisation and transport patterns, as there is growing evidence that pollen is subject to long-distance atmospheric transport (Sofiev 2017).

Knowing the pollen sources and concentration, phenological data of the areas surrounding the pollen collection locations, contribution of non-local wind-pollinated species and patterns of distant pollen air masses and influence of climate are all essential parameters of a reliable pollen forecasting system (Davies et al. 2017). One of the most widely used models that has been used as a pollen forecasting system in Europe is the SILAM dispersion model (Sofiev 2017; Prank et al. 2013; Sofiev et al. 2013; Ranta et al. 2006). The essential inputs for the SILAM dispersion model are atmospheric transport processes including transport with wind (advection), turbulent mixing (diffusion) and grain removal by wet and dry deposition, as well as meteorological data taken from weather prediction models (Sofiev et al. 2015; Sofiev 2002; Kouznetsov and Sofiev 2012). Pollen emission data in SILAM relies on input from the extensive pollen maps that cover the European continent (Sofiev 2017). Use of remote sensing capabilities can identify grassland sources and grassland greenness indices as a measure of seasonal fluctuations in grassland biomass based on the specific wavelength at which plants absorb light (Ma et al. 2015; Luvall et al. 2011). This information can be fed into pollen transport and dispersion models. Airborne transport of pollen is an important factor in Australia, therefore it is desirable to explore the options for remote sensing and model (e.g., SILAM) implementation in Australia.

23 Consultation and Stakeholder Engagement

The development of this Standard involved broad and varied consultation and engagement. Feedback on the initial draft was sought by email from those listed in the Acknowledgements. A second round of feedback was also sought 19 March- 27 April 2018 on Version 1.1.

Consultation and engagement also took place at the following event:
NHMRC AusPollen Partnership Investigator Workshop, 1-3 June 2017, Centre for Children's Health Research (QUT), South Brisbane.

24 Schedule for Review of Standard

Standards are living documents which reflect progress in science, technology and systems. To maintain its currency, this Interim Australian Standard and Protocols document will be periodically updated and reviewed, and new editions published. Between editions, amendments may be issued. Standards may also be withdrawn (Standards Australia and Standards New Zealand 2016). This Standard and Protocols document will be reviewed annually over the next three years from the date it was first released, and thereafter, every 5 years. The information on standard approval, commencement, amendment and review are listed in Table 8. The review process will be initiated and undertaken by some or all of the original authors and additional authors may be invited to contribute to the revision of the Standard and Protocols.

We welcome suggestions for improvement for this Standard, and especially encourage readers to notify us immediately of any apparent inaccuracies or ambiguities. Please address your comments to the corresponding author via email to paul.beggs@mq.edu.au.

Table 8: Standard approval, commencement, amendment, and review information.

Date Released Version 1.0	27 September 2017
Date of Commencement	1 October 2017
Amendment History	Interim document, Version 1.0
	Interim document, Version 1.1 (1 November 2017)
Reviewed 2018, expert reference group	Interim document, Version 1.2 (19 March 2018)
Current amended version	Published Interim document Version 2.0 (14 September 2018)
Standard(s)/Guideline(s) Superseded by this Standard	Hasnain SM, Katelaris CH, Newbegin E, Singh AB (2007) Aeroallergen Monitoring Standard for The Asia Pacific Region: A WAO manual for the use of the Burkard Volumetric Spore Trap and Burkard Personal Volumetric Air Sampler. World Allergy Organization.
Keywords	Pollen; Grain; Fungal; Spore; Aerobiology; Aeroallergen; Monitor; Sample; Standard; AusPollen; Network; Australia; National

25 Authorisation

This Standard and Protocols document was prepared by investigators of the AusPollen Aerobiology Collaboration Network, an NHMRC funded AusPollen Partnership Project (1117107) in conjunction with and in consultation with a panel of national and international experts in various aspects of pollen and spore monitoring and forecasting (see Section 2 Acknowledgements). The AusPollen Partnership has a core objective to establish and implement nationally standardised processes for collection, counting and reporting of airborne pollen and spore concentrations in Australia. This Interim Standard and Protocols document will be initially released for publication by the AusPollen Partnership for use by the broader AusPollen Aerobiology Collaboration Network. Once the joint Working Group for AusPollen Aerobiology Standard is established through the Australasian Association for Aerobiology and the Australasian Society of Clinical Immunology and Allergy, and the Terms of Reference have been accepted by both organisations, this Standard and Protocols document will be formally accepted by these two relevant peak professional organisations.

26 Appendices

26.1 Height of Volumetric Sampler

A number of studies have investigated differences in airborne pollen and spore concentration at elevated and at ground level and contradictory results have been observed. Most of the studies recognised the sampling height as an important factor that influenced the pollen and spore concentration (Khattab and Levetin 2008; Alcázar and Comtois 2000), however some studies have suggested its limited impact on bioaerosol levels (Fernández-Rodríguez et al. 2014). The peak pollen values at greater heights were observed later than those at the ground level (Galán et al. 1995a; Hart et al. 1994; Rantio-Lehtimäki et al. 1991; Käpylä 1981). It was suggested that airborne pollen and spore monitoring sites should be located above ground level to avoid the direct influence of local vegetation and anthropogenic sources as well as to better sample the regional pollen flux that is dominated by major wind-dispersed pollen taxa (Haberle et al. 2014). However, pollen and spore monitoring performed using a sampler located at high elevated surfaces can also negatively influence the sampling concentrations. For instance, pollen levels from a 30 m spore trap were found to be consistently smaller than those from 12 and 24 m traps for all types examined and the suggested reason was that pollen from local sources was not sufficiently mixed in the air to reach the 30 m trap (Hart et al. 1994). Moreover, Rantio-Lehtimäki et al. (1991) suggested that reliable monitoring can be performed at an elevated high level of tree pollen species but not the pollen of herbal plants, which includes allergenically important species and further emphasised the importance of monitoring on both low and elevated heights.

In Australia, grass pollen is the main outdoor source of aeroallergens that trigger hay fever and allergic asthma (reviewed by the ACEAS Working Gorup; Davies et al., 2015). Rantio-Lehtimäki et al. (1991) have observed delayed grass pollen detection (1-2 weeks) at roof level compared to ground level and has suggested that sampling should be done at a low level especially at the beginning of the flowering season.

26.1.1 Location (Including Sampler Height) Recommended by Other Standards

Standardised Protocols established by the Pan-American Aerobiology Association (Rogers and Muilenberg 2001) suggest the rooftop as a suitable sampler location with the following suggestions for sampler installation:

- Platform-height to be 5-15m above the ground centrally on the rooftop;
- As far from trees as possible;
- Positioned with no interference between ambient air flow and rooftop structures (should not be more than 20° higher than sampler inlet level;
- If parapet present, sampler inlet level should be raised at least 76.2 cm above it.

European Committee for Standardization created the technical specification for monitoring the airborne pollen and spores for allergy networks (European Committee for Standardization 2015) and had listed the following requirements for sampler positioning:

- It should be on the roof of a building and away from the edge (ideally 2m or more);
- Positioned to ensure that adjacent buildings do not screen the sampler or interfere with the ambient air flow;
- The height depends on the city and on the height of neighbouring buildings;
- Elevated between 100 cm to 150 cm from the roof.

26.2 Safety Data Sheets

Polydimethylsiloxane:
https://www.sigmaaldrich.com/catalog/substance/polydimethylsiloxane123456314862911?lang=en&region=AU
Sylgard 527:
http://www.dowcorning.com/applications/search/default.aspx?R=229EN
Basic fuchsin:
https://www.sigmaaldrich.com/catalog/product/sial/215597?lang=en&region=AU
Phenol:
https://www.sigmaaldrich.com/catalog/substance/phenol941110895211?lang=en&region=AU
Cyclohexane:
https://www.sigmaaldrich.com/catalog/product/aldrich/320633?lang=en&region=AU

26.3 Minimum Standards for Daily Pollen Monitoring for the Victorian Thunderstorm Asthma Pollen Surveillance (VicTAPS) Network

The specific project-related requirements for the Victorian Thunderstorm Asthma Pollen Surveillance (VicTAPS) Network are detailed in Table 9. Compliance with these minimum standards will be managed via the Quality Control of counting and site aAuditing detailed in Section 13 Quality Control and Auditing and Section 26.9 Pollen Monitoring Site Audit Checklist.

These VicTAPS minimum standards were prepared in consultation with Dr Fay Johnston (University of Tasmania).

Table 9: Minimum Standards for Daily Grass and Total Pollen Surveillance for the VicTAPS Network.

Aspect	Minimum Requirement
Sampler Position	A sampler must be placed on a readily accessible, flat, horizontal surface, following the minimum location criteria: • Height above the ground: 1.5 – 15 m; • Minimum 1m vertical and 2m horizontal distance from any protruding supporting structures; • Sampling inlet with minimum clear sky angle of 120°; • Unrestricted airflow of 270° around the sampling inlet orifice.
Flow Rate	Continuous sampling with flow rate of 10 L/min.
Flow Control	Checked weekly with the flow meter (Burkard Manufacturing).
Monitoring Period	1 October – 31 December.
Slide Changeover Time	9 am.
Pollen Concentrations and Forecasts Available on AusPollen Data Submission Portal for the Bureau of Meteorology's Forecast	11 am.
Slide exchange log	Entry of details of the slide exchange in a data entry log or booklet (see section 10.6 on record keeping and Appendix 26.4)
Adhesive	Silicone (polydimethylsiloxane) based.
Mounting Media	Glycerine, gelatine or polyvinyl alcohol (e.g., Gelvatol).
Staining	Fuchsin (e.g. Calberla's stain contains basic Fuchsin, see Appendix 26.5)
Surface Examined	2 x longitudinal (horizontal) transects equidistant from edges of the tape and each other.

Counting Magnification	40x objective and 10x ocular lens (400x magnification).
Taxa reported for surveillance	Grass pollen and total pollen collected and counted daily.
Conversion Factor	Atmospheric pollen or fungal spore concentrations should be expressed as the daily average pollen grains or fungal spores per cubic meter of air. This can be calculated using the following equation, which takes into account all the relevant factors. $$[\text{Pollen or Spore}] = n\left[\frac{L \times W}{L \times (\frac{F}{M}) \times N} \times \frac{1}{D \div 1000 \times t}\right]$$ where [Pollen or Spore] - atmospheric pollen grain or fungal spore concentration per cubic metre n - number of pollen grains or fungal spores counted in the analysed area of the microscope slide L - length of impaction area on the melinex tape or glass slide (mm) e.g., 48 mm W - width of impaction area on the melinex tape or glass slide (mm) e.g., 14 mm F - field number written on the eyepiece (typically a number between 18 mm and 25 mm) M - objective magnification (40) N - number of transects e.g., 4 D - flow rate of the equipment (l/min) e.g., 10 l/min t - duration of the sampling period (min) e.g., 1440 min
Site Audit	Site participation in annual site audits (see Section 13.1 and Appendix 26.9)
Training and quality control of counting	Counters will participate in training and consider quality control of counting processes including certification of counting proficiency (Section 11 and 13).

26.4 Sampling Logs from Some of the Australian Pollen and Spore Monitoring Groups

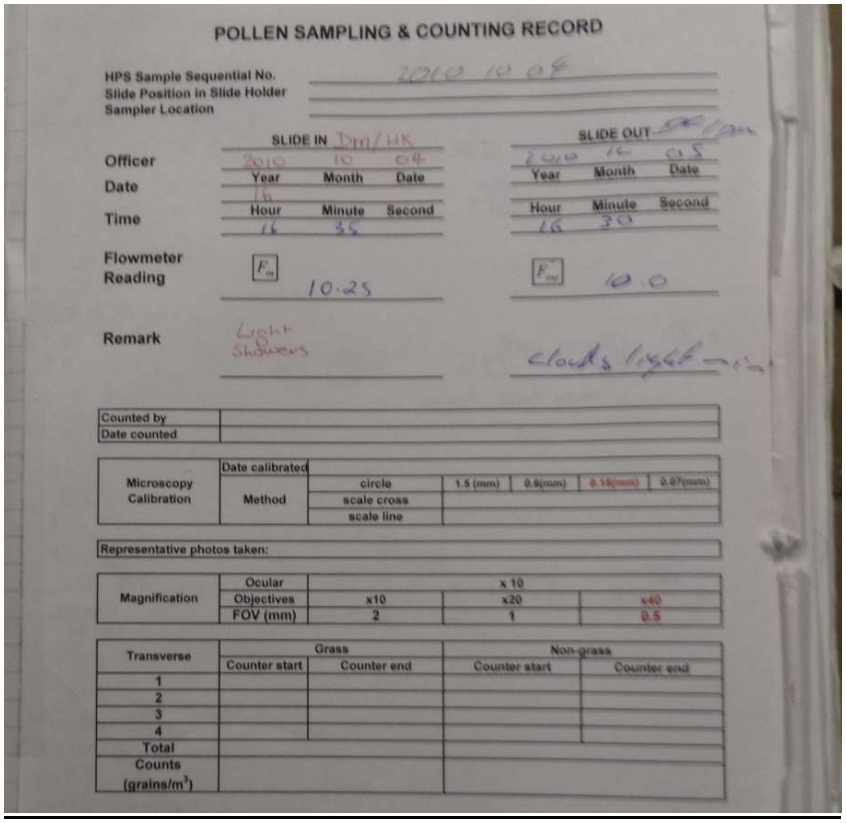
Rocklea data logging (Queensland University of Technology)

Pollen and spore monitoring record sheet:

- Set up date
- Operator
- Set up/slide exchange time
- Flow rate
- Code used on drum/slide
- Number full collection days
- Harvest comments

Pollen and spore slide record sheet:

- Date of first day
- Code on slide
- Burkard instrument number
- Slide number
- Time of day 1 set up
- End time day 7
- Site code
- Burkard operator
- Person who mounted slide
- Date slide mounted
- Comments



POLLEN SAMPLING & COUNTING RECORD

HPS Sample Sequential No. 2010 10 04
Slide Position in Slide Holder
Sampler Location

	SLIDE IN DM/HK			SLIDE OUT		
Officer						
Date	2010	10	04	2010	10	05
	Year	Month	Date	Year	Month	Date
Time	16					
	Hour	Minute	Second	Hour	Minute	Second
	16	35		16	30	
Flowmeter Reading	F_{in} 10.25			F_{out} 10.0		
Remark	Light showers			clouds light		

Counted by	
Date counted	

Microscopy Calibration	Date calibrated				
	Method	circle	1.5 (mm)	0.6(mm)	0.15(mm) 0.07(mm)
		scale cross			
		scale line			

Representative photos taken:

Magnification	Ocular	x 10		
	Objectives	x10	x20	x40
	FOV (mm)	2	1	0.5

Transverse	Grass		Non-grass	
	Counter start	Counter end	Counter start	Counter end
1				
2				
3				
4				
Total				
Counts (grains/m^3)				

Figure 12: Pollen sampling and counting record from Dr Danielle Medek.

Recording sampling and collection logs from Dr Penelope Jones for the AirRater Project

Collection

- Site code
- Date and time of collection

Counting

- Date the pollen slide represents
- Date and time of pollen count entry
- Person responsible
- Raw counts (25 key taxa, plus 'other/unknown')
- Any free comments: readers note here any issues with the slide, unusual weather conditions, high amounts of debris on the slide etc.

Reporting

- Conversion of raw counts into grains/m^3 following Rogers and Muilenberg (Rogers and Muilenberg 2001)
- Total pollen count that includes all pollen taxa + other/unknown, but excludes *Alternaria*
- *Alternaria* is reported in the app but kept separated

26.5 Preparing Calberla's stain (The University of Melbourne Undated)

Information provided by Associate Professor Edward Newbigin (The University of Melbourne)

Calberla's stain contains basic Fuchsin and stains pollen grains pink, but does not stain fungal spores or most of the other debris that is usually present on slides. Basic fuschin is available in a powder form from a number of suppliers. Only a very small amount is needed to make up the saturated aqueous basic fuschin. Some suppliers sell the stain in a ready-to-use form.

Procedure: Mix together a 50:50 solution of Fuschin (saturated aqueous basic Fuchsin; https://www.sigmaaldrich.com/catalog/product/sial/215597?lang=en&region=AU) and glycerine. Upon cooling, the solution will form a dark pink gel. Store the solution in an airtight jar for future use. Mix the glycerine (5 ml), ethanol (95% (v/v); 10 ml), distilled water (15 ml) and 6 drops of the melted fuschin/glycerol solution. Store the solution in a clear bottle with a dropper lid.

26.6 Summary of Metadata Fields for the NEMSR

Content of Tables 9 to 11 were prepared by Dr Andre Zerger (Bureau of Meteorology, Canberra) and Chacko Thomas (Bureau of Meteorology) in consultation with members of the AusPollen Partnership particularly Professor Janet Davies and Dr Edwin Lampugnani (The University of Melbourne).

Table 10: NEMSR metadata fields for network information.

Field name	Description	Mandatory?	Vocabulary?	Example
dataProvider	Name of data custodian	Y	N	Academic institution name
id	The unique identifier of the network (created by the NEMSR team)	Y	Y	
name	The name of the network	Y	N	The Australian Pollen (AusPollen) Allergen Network
networkDescription	The description of the network	Y	N	A standardised and certified network of Burkard volumetric samplers used to monitor airborne pollen and fungal spore concentrations
networkURL	A URL containing a resource with information about the network	Y	N	http://www.pollenforecast.com.au/
environmentalTheme	The primary environmental theme of the network	Y	Y	Air
contactDetails/name	The name of the contact for the network	N	N	Data Coordinator
contactDetails/phone	The phone number of the contact for the network	N	N	
contactDetails/address	The address details of the contact of the network	N	N	Address of institute
contactDetails/onlineResource	The contact email address or a URL containing contact details	N	N	Email address of key contact

Field name	Description	Mandatory?	Vocabulary?	Example
Funding source	Funding source(s) that support the pollen monitoring site	Y	Y	This site is supported by the National Health and Medical Research Council AusPollen Partnership Project Grant 1116107 OR Australian Research Council Discovery Project Grant DP170101630 OR AirRater OR VicTAPS OR other as appropriate

Table 11: NEMSR metadata fields for site information.

Field name	Description	Mandatory?	Vocabulary?	Example
id	A unique identifier for the site	Y	N	P1
name	The name of the site	Y	N	Parkville 1
siteDescription	A short description of the site	N	N	The primary pollen count station for the _____ region
siteURL	A URL for the data or further information	N	N	http://www.melbournepollen.com.au/
siteLicence	The type of licence for the site metadata	Y	Y	Creative Commons, Attribution- Non-commercial, ShareAlike attribution 3.0 Australia (CC BY-NC-SA 3.0 AU)
srsName	The EPSG code of the spatial referencing system used to locate the entity	Y	Y	
longitude	The longitude of the site	Y	N	144.96489
latitude	The latitude of the site	Y	N	-37.79713
Elevation (ahd)	The elevation of the site (Australian Height Datum)	N	N	
operatingAuthority/name	The organisation that is the operating authority for the site	Y	N	The University of Melbourne
operatingAuthority/url	A URL for a webpage or resource with information about the operating authority	N	N	http://biosciences.unimelb.edu.au/
siteStatus	The operating status of the site	Y	Y	Active
Archive data	URL for access to archived weekly data	N	N	http://aceas.tern.org.au/knb/metacat?action=read&qformat=html&docid=aceasdata.25.7

Table 12: NEMSR metadata fields for observing capability information.

Field name	Description	Mandatory?	Vocabulary?	Example
observedProperty	The environmental phenomenon (property) being measured at a site	Y	Y	atmosphere-Aerobiologicals
procedure	An external link to the procedure used to make the site observations	N	N	To be advised (will link to new Australian Aeroallergen Monitoring Network Standard and Protocols)
observationMethod	The method used to make observations	Y	Y	inSituLandBasedPlatforms
dataAvailabilityStatus	The availability of the observation data	Y	Y	available-online
samplingRegime	The frequency of site observations	Y	Y	oneDaily
firstObservationDate	The date of the first observation at the site	N	N	2009-12-19
finalObservationDate	The date of the last observation at the site	N	N	ongoing
Seasonal duration of counting	Months of the year that monitoring occurs	Y	N	October – March or as appropriate
Pollen taxa counted and reported	Botanical family names of pollen grains counted	Y	Y	List taxa counted
Fungal spores reported	Genus names of fungal spores counted	N	N	List taxa counted

26.7 Other Network Quality Assurance and Standardisation Studies

(Galán et al. 2014):
Participants: Total of 45 technicians from 15 different countries
Exercises: Two slides from different geographical region
Slides and taxa: Moderate amount of pollen (>40 grains/m^3 and <100 for grasses and <200 for olive or birch); For sites in Northern Europe sample prepared by Medical University of Vienna with one with birch and second one with grass pollen (34 technicians) and for sites in Southern Europe sample prepared by University of Córdoba one with grass pollen and other one with olive (11 technicians);

(Oteros et al. 2013):
Participants: 25 participants from Spain
Exercises: 3 slides from different seasons (winter, summer and spring)
Slides and taxa: The same pollen types for all: Amaranthaceae, *Alnus*, *Fraxinus*, Cupressaceae, *Pinus*, *Populus*, *Platanus*, *Betula*, *Quercus*, *Morus*, *Urtica*, *Olea*, Poaceae, Castanea, *Rumex*, *Plantago* and total pollen count.
(Sterling et al. 1999)

2 sites

Daily slides from the month of September (1996)

2 counting techniques

26.8 Australian Airborne Pollen Data Repository Proposal (for the National Computational Infrastructure repository) (August 2017)

Document prepared by Dr Edwin R. Lampugnani (The University of Melbourne), Dr Beth Ebert (Bureau of Meteorology) and Professor Janet Davies in consultation with the AusPollen Aerobiology Collaboration Network.

Scope

Dataset title: Australian aeroallergen data repository provided by a network of aeroallergen monitoring sites across Australia.

Background

This dataset consists of historical daily or weekly aeroallergen concentrations from a network of aeroallergen monitoring stations across Australia. The most common method of daily aeroallergen monitoring in Australia is based on the deployment of a Hirst-type continuous flow volumetric pollen and spore trap. Count methods used in each location comply with the Standards Australia and Standards New Zealand (2016).

Pollen and fungal spore traps are calibrated to sample airborne pollen and fungal spores with flow rates at 10L per minute. Since grass pollen constitutes approximately 18-23% of aeroflora throughout most of Australia, and due to the high prevalence of grass pollen allergy, grass pollen concentrations are determined by collation of light microscopy counts from longitudinal transects and recorded separately from other taxa. Microscope slides are retained for future assessment of other types of airborne biologicals if necessary. Quality control of the counting processes includes inter-laboratory evaluation.

This implementation was developed by the AusPollen Aerobiology Collaboration Network and is supported by a number of academic institutions, non-government organisations, and government institutions including the Australian Bureau of Meteorology (BoM) and National Computational Infrastructure (NCI). The dataset includes pollen concentrations collected from more than 5 stations across Australia, with some sites spanning more than 20 years.

The current procedure for authorising access to the aeroallergen concentration data is handled by the AusPollen Aerobiology Collaboration Network. Expressions of interest in obtaining access to the data should be sent by email to AusPollen@qut.edu.au

Benefits

Aeroallergen monitoring and forecasting is a critical component to the development of robust forecast models and warning systems that can advise the millions of Australians with hay fever and/or asthma

of days with high levels of pollen exposure and critically are an essential component of developing a thunderstorm asthma forecast.

Responsibilities

AusPollen will continue to maintain and supplement the aeroallergen dataset over the next 2 years.

Infrastructure

Overview of data hosting, portals and community workflow is given in Figure 9.

Data Providers

Individual aeroallergen monitoring stations can elect to be part of the Australian Aeroallergen Monitoring (AusPollen) Network and supply information for data storage and distribution to external researchers. At this time, the network consists of aeroallergen monitoring stations at Melbourne, Canberra, Sydney and Brisbane which are coordinated by academics from The University of Melbourne, The Australian National University, Western Sydney University and Queensland University of Technology, respectively. Additional data providers can request inclusion by emailing auspollen@qut.edu.au

Data Amalgamator

AusPollen provides a framework for aeroallergen monitoring station operators to submit aeroallergen concentrations for archival and subsequent distribution to data hosting providers and collaborative academics for research purposes.

Data Hosting

Hosting infrastructure will be provided by NCI through the 10 PB RDS national data collection. This collection, maintains datasets of national significance from the fields of astronomy, ecology, geology, meteorology, climatology, and oceanography. Data access is provided using THREDDS using the ISO 19115 geospatial metadata schema.

Data Portals

A number of portals will harvest metadata from NCI to provide visibility and accessibility across various user groups. This includes Terrestrial Ecosystem Research Network (TERN) for ecosystem science and management, Australian National Data Service (ANDS) for government and research institutions (including data.gov.au) and

National Environmental Information Infrastructure (NEII), a portal service for nationally significant datasets.

Usage

In addition to research use, daily pollen measurements are provided free of charge to subscribers in Melbourne, Sydney, Canberra and Brisbane. The BoM is developing a pilot pollen and thunderstorm asthma forecast service for the Victorian Department of Health and Human Services that will use pollen data to initialise and verify the pollen forecasts.

Ongoing engagement with the community through workshops, conferences and software toolkits will be used to promote additional usage of the data on the NCI archive to the wider community of academic researchers.

Attribution and License

A Creative Commons (CC) 3.0 Restrictive License Agreement license will be applied to the aeroallergen dataset. This license will restrict commercial applications and ensure the license is transferred onto derived products. Furthermore, the NCI dataset will not be published, but instead require researchers to request access to the dataset. This is an additional mechanism to discourage commercial pollen forecasting applications.

Attribution to the original data provider, the AusPollen Aerobiology Collaboration Network, its network partners, funding sources and the Australian Bureau of Meteorology will be required for publications. The AusPollen logo and attribution details will be provided as part of the NCI metadata table.

Costs

Historical aeroallergen concentrations were provided by the AusPollen Aerobiology Collaboration Network partners at no cost for non-commercial use. Initial establishment and transfer costs from AusPollen to NCI will be covered as part of the Bureau of Meteorology's investment in the NCI. Subsequent costs for updating the NCI from AusPollen will also be covered by the Bureau's investment.

26.9 Pollen Monitoring Site Audit Checklist

Developed by Professor Janet Davies and Dr Andelija Milic (QUT).

Audit site		**Audit date**
Host Organisation		
Address		
Site representative present at audit	**Name:** **Role:**	**Signature:** **Date:**
Auditor	**Name:** **Role:**	**Signature:** **Date:**

Audit Scope

The site audit will check the set-up of the pollen monitoring site and the processes for monitoring airborne concentrations of pollen. The audit will not focus on work health and safety practices associated with each site as this remains the responsibility of the host organisation.

The Site Audit Checklist has been designed to systematically collect information on all sites and it is based on the recommendations and minimum standards described in the Australian Airborne Pollen and Spore Monitoring Network Interim Standard and Protocols, and in particular the processes described in the VicTAPS (Appendix 26.3), and VicTAPS training module.

Check list criteria:

1. *Monitoring site location*
2. *Pollen and spore trap position*
3. *Pollen collection date and time*
4. *Slide/drum preparation, mounting and daily counting processes*
5. *Pollen concentration conversion*
6. *Quality Assurance of the counting process*
7. *Risk assessment and safety*
8. *Process logging and data management*
9. *Communication and use of pollen information*
10. *About the counters*

Audit Outcomes

An Audit Report will be generated for each site based on the check list in this document. The report may include comments or recommendations and timelines for any improvements to the processes.

The Site Audit Report will be provided to the named organisation responsible. An Audit Report Summary including individual Site Audit Reports will be provided to the organisation requesting the Site Audit.

The decision to adopt and implement any recommendations, and timeframes for implementing changes, of the Audit Reports will be the shared responsibility of the organisation requesting the Site Audit, the organisation responsible for the site and the appropriate oversight committee or working group.

Audit Checklist

1. Pollen and spore monitoring site location

Questions	Yes	No	Comments
1.1 What is the physical location of the trap (building)?			
1.2 Does the site location meet the requirements and aims of the project (site monitoring)?			
1.3 Is the site (A) urban, (B) peri-urban or (C) rural?			
1.4 Are air quality and/or meteorological monitoring sites within the vicinity? (note name and location)			
1.5 Is the site secure and safe? (low risk for personnel, regulatory requirements; from vandalism and natural disasters)			
1.6 Is site easily accessible? (for collection and transport of the equipment)			
1.7 Is adequate service to the site provided? (e.g. continuous electricity supply)			

2. Pollen and spore trap position

Questions	Yes	No	Comments
2.1 Is sampler positioned between 1.5 to 15m above the ground? (note exact height)			
2.2 Is sampler located on (A) ground, (B) roof top, (C) platform, (D) brackets, or (E) other?			
2.3 Is the sampler positioned flat on a supporting surface?			
2.4 Is the sampler Hirst type volumetric sampler? (Note sampler supplier)			
2.5 Is the sampler properly anchored?			
2.6 Is the sampler at least 1m vertical and 2m horizontal from any protruding supporting structures?			
2.7 Does the sampling inlet have a minimum clear sky angle of 120°?			
2.8 Is the airflow unrestricted around the sampling inlet orifice? (free from obstructing trees and buildings)			
2.9 Is there any additional equipment used for			

accessing the sampler? (e.g., stairs, ladders, step stools, platform lift etc.)			
3.0 What type of flow meter is used?			
3.1 Is continuous sampling with flow rate of 10 L/min checked? How often?			

3. Pollen collection date and time

Questions	**Yes**	**No**	**Comments**
3.1 Is the minimum monitoring period ensured? (note dates)			
3.2 What is the duration of daily monitoring? (note dates)			
3.3 Is daily counting performed during the grass season?			
3.4 Is the sampling time consistent? (If so, note sampling time. If not, note variation of the sampling time)			
3.5 If the site is reporting pollen counts, what time are they available? (e.g. 11am for VicTAPS project?)			
3.6 If a 7-day sampling drum is used and weekly sampling performed, what is the drum change day?			

4. Slide/drum preparation, mounting and daily counting processes

Questions	**Yes**	**No**	**Comments**
4.1 What kind of slide or drum adhesive is used?			
4.2 How are slides labelled? (e.g. site code or colour, date)			
4.3 What kind of stain solution is used?			
4.4 Are the mounted slides directly counted by light microscopy?			
4.5 What kind of microscope is used?			
4.6 Is the microscope magnification of 400x applied (40x objective and 10x ocular lens)? (If not what is the magnification applied)			
4.7 Is the longitudinal (horizontal) counting performed?			
4.8 Is minimum surface examined 10% of the whole			

slide deposition area? (If no, note whether this counting is for daily grass and total pollen surveillance)			
4.9 How many longitudinal (horizontal) transects are examined; 3, 4 or more, or other?			
4.10 Where and what facilities are used for preparing the slides for sample collection, mounting and counting?			
4.11 What taxa are counted?			

5. Pollen concentration conversion

Questions	**Yes**	**No**	**Comments**
5.1 Is grains/m^3 of air used as a unit of concentration of biological particles?			
5.2 Is the site specific conversion factor used for conversion of raw counts to concentration?			
5.3 What is the site-specific conversion factor used?			
5.4 What is the field of view (40x objective)?			

6. Quality Assurance of the counting process

Questions	**Yes**	**No**	**Comments**
6.1 Is quality assurance of slide counting undertaken?			
6.2 Who conducts the QA counting of slides?	Organisation: Site: Person (if known):		
6.3 If QA in conducted by your site, is there a record of undertaking the QA counting?			
6.4 If the QA is not done at your site, when and how often are slides transported to the lab that does the QA counting?			
If QA counting is conducted at this site, complete the following questions			
6.5 For which sites does this laboratory perform the QA of slide counting?			
6.6 How long after the daily grass and total pollen			

counts are the QA counts of slides conducted?			
6.7 What kind of microscope is used for QA?			
6.8 Is the microscope magnification of 400x applied (40x objective and 10x ocular lens)?			
6.9 What is the site-specific conversion factor used?			
What is the field of view (40x objective)?			
6.10 Is the longitudinal (horizontal) counting performed?			
6.11 Is minimum surface examined 10% of the whole site deposition area?			
6.12 How many longitudinal (horizontal) transects are examined; 3, 4 or more, or other?			
6.13 What taxa are counted?			

7. Risk assessment and safety

Questions	**Yes**	**No**	**Comments**
7.1 Has the site undertaken a risk assessment? (risks and mitigation, material safety data sheets for monitoring including monitor installation, and slide collection/mounting/counting)			
7.2 Have all individuals involved in pollen and spore monitoring signed the risk assessment?			
7.3 Are material safety data sheets for chemical involved available and accessible?			
7.4 Have all individuals involved in pollen and spore monitoring lab work (mounting and counting) used Personal protection equipment?			

8. Process logging and data management

Questions	**Yes**	**No**	**Comments**
8.1 Does the site have a process for logging the collection of airborne pollen and spore samples? (Sampling log)			
8.2 Does the site have a process for logging the processing, storage and transport of pollen slides			

(slide register)?			
8.3 Does the site use a daily pollen counting record sheet?			
8.4 What data management processes are used? (paper or digital)			
8.5 Where are the following primary records retained	i) the sample log: ii) the slide register: iii) the count records:		

9. Communication and use of pollen information

Questions	**Yes**	**No**	**Comments**
9.1 How is the pollen count information disseminated? (website/App)			
9.2 Is a pollen forecast provided? If so, for how many days?			
9.3 What organisation is responsible for and owns the pollen count data?			
9.4 How are the daily pollen data stored? (server name, server location; e.g. NCI daily data)			
9.5 What are the projects that this pollen information contributes to? (e.g. VicTAPS, AusPollen, ARC DP, AirRater)			
9.6 How is the seasonal pollen data archived? (e.g. TERN portal)			
9.7 Is this data publicly accessible and available for additional use? (under what conditions e.g. license)			
9.10 Is information about this site accessible and publicly searchable? (i.e. NEMSR)			

10. About the counters

Register of counters and contributors (list all personnel for this site who contribute to pollen monitoring)	**Primary occupation/position** (e.g. student, technician, facilities manager)	**Role in project** (e.g. pollen counter, coordinator)	**Pollen aerobiology training undertaken** (e.g. VicTAPS training, online training, one on one, workshop)

Comments and Recommendations

Recommendations with timeframes for implementing changes

27 References

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