Guidelines on the preparation of biological materials for accessioning into long-term collections

Contents

1 Collections

1.1 How do collections serve biosecurity?

Scientific collections are foundational to biological research. Natural history collections were established to document and display the diversity of life on earth – storing reference specimens as species were discovered and described. Modern research tools such as DNA analysis from preserved specimens mean that the information contained in collection has significantly increased in value for research applications.

Retention of voucher specimens from biosecurity diagnostics is essential for meeting international reporting requirements under International Standards for Phytosanitary Measures No. 17. Biosecurity collections across Australia form the basis of official pest and disease records for our country, and our collection data is shared via the Australian Plant Pest Database. Collections are internationally recognised repositories that underpin all biosecurity-related activities. Retention of physical specimens and their data allows us to re-visit our records, inform epidemiological studies, validate diagnostics, support surveillance, write legislation and regulations, conduct pest-risk analyses and support trade and market access for both importers and exporters. Collections are a valuable resource for agricultural and biosecurity research and serve as a genetic biobank that we have only just begun to tap into.

It is essential to lodge the physical evidence behind significant detections of pests and diseases and to continue to document the occurrence of these organisms as they become established. For a scientific collection to be successful it relies on consistent submissions of high-quality specimens from the scientific community. Collections are a dynamic repository that are continually revised, added to, and improved. This process ensures that Australian plant pest and disease records remain accurate and current. Providing specimens to collections makes them better equipped to continue to support biosecurity and market access activities.

Goal 1 	Standardise the accessioning of specimens into collections
Goal 2 	Educate collectors on specimen preservation techniques
Goal 3 	Improve collection processes and optimise the accessioning workflow

This guide has been prepared to assist you in the preparation of biological samples for long-term storage, ensuring their successful preservation for future study, with the aim that specimen vouchering becomes standard practice for diagnostics, research, and biosecurity surveillance.

The practices proposed here are designed to give you an overview of preservation methods for plant pest and disease collections. By educating stakeholders on these practices we hope to provide a better service to the scientific community by optimising collection processes and workflows.

1.2 What are scientific specimens?

A specimen can be a new species to science, species distribution and temporal records, new host records, samples generated during research, surveillance, or diagnostic activities (including quarantine interceptions) or samples that will update our collections by providing appropriately preserved material for molecular and other analytical methods.

Biosecurity collections across Australia hold a variety of organisms both directly or indirectly related to biosecurity and agroindustry. While the focus is on pests and diseases of plants or animals these collections can also include diseases of pests or beneficial insects, diseases of fungi, food industry organisms, environmental pests (e.g. ants, termites), non-pathogenic organisms associated with plants (e.g. bees, pollinating beetles, fungal endophytes) and fruiting bodies of macro-fungi. All these specimens better inform our understanding of species concepts and the biology of organisms which informs biosecurity preparedness and response.

Specimens can take several forms. This includes pressed and dried plant material, pinned insects, slide mounted invertebrates and microbes, specimens preserved in fixative and even living cultures. Specimens can be whole or part and include items like DNA and photographs. Specimens often also include notes from the collector or identifier, or the author of the species. These peripheral materials augment these collections into historical accounts and add historical value.

1.2.1 What are scientific specimens used for?

Specimens are used for a variety of purposes, a few include:

- allow and support accurate identification of species
- provide a permanent record for a species occurring at a particular time and place
- document the introduction and spread of invasive species over time
- the reference point for the application of scientific names
- provide biological material for taxonomists, ecologists, and other researchers
- serve as vouchers for biosecurity reporting and area of freedom claims

1.3 Points to consider before preserving specimens

1.3.1 Plan ahead

Methods optimised for field surveillance or collection of specimens for diagnostics may not be appropriate for other applications or long-term storage. Consider if the effort gone into collecting and identifying a sample makes it worthy of long-term preservation, and how specimens may be used in the future. What other things need to be considered with your sample handling and what collection data is captured and how is this stored and accessed? How will specimens be supplied to the collection? How are specimens stored before vouchering? All these steps affect specimen suitability for accessioning into collections.

1.3.2 Fresh is best

If you are not preserving the sample yourself then it must be sent to the collection as fresh as possible (but not alive!). Posting samples in ethanol or frozen (for arthropods) or chilled and with ice

bricks (for fungi) for example. This is dependent on the biosecurity status of the sample and species-specific requirements.

1.3.3 Host material is crucial

Host material is vital in the study of both plant pests and diseases. For collections, having host material strengthens the confidence of record details and allows for future confirmation of the host identification. It also provides examples of symptoms and damage. When collecting host material, include more than the diseased/damaged material, making sure to collect flowers and fruit if available, and whole stems with leaf arrangement. Take sufficient photos for examination. You will also need to consider biosecurity status of the host plant or endangered/threatened status for collecting and moving material.

1.3.4 Collecting permits

Permission or collecting permits are always required when collecting specimens. Even on public land, the local government area council should be contacted for permission. For private land the owner must be consulted. For state and national parks, a scientific collecting license must be obtained and presented to the local park manager for approval.

1.3.5 Data management

Data management starts at the time of collection. Ensure thorough documentation of collected specimens from collection right through the diagnostic processes until curation. Avoid using short cuts such as short-hand, codes, acronyms, and initials. Have a data management strategy for sampling. You can pre-print forms to ensure all necessary data is captured. A list of suggested collection data has been included here in section 321 Collection data and peripheral materials.

Data privacy and data sharing approvals should also be considered when providing information to a collection. Is the specimen from a diagnostic lab, does the submitter form or policy explain that diagnostic samples and collection data may be vouchered in a collection and shared with online data providers? You could give the submitter the option to share or exclude their name and address, providing the collection with deidentified collection data.

1.3.6 Cost of accessioning

Collections have offered their services free of charge for centuries with the understanding that these specimens are made available to the greater scientific community, it is a public service and has been supported by public funds. With the increasing requirement to voucher specimens for publishing, and talk of specimen management plans from funding bodies, collections are predicting increasing demands on our vouchering services.

We strongly recommend that researchers consider the costs involved in accessioning their research material and factor this into budgets at the project proposal stage, as they would with any other research services they use, such as data storage or bioinformatics consulting. To aid in this practice we have included here guides to average costs to accession different types of specimens based on data from 2022/2023 financial year. See section **Error! Reference source not found. Error! Reference source not found.**.

2 Recommended practices for preservation

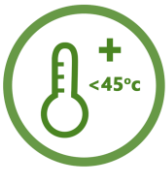
> ! For all preservation methods described here it is critical that specimen data is retained with the specimen throughout the process.

2.1 Dried material - Plant (diseased or host)

2.1.1 Equipment

- Herbarium press or heavy flat object(s)
- Newspaper and Cardboard
- Drying oven (optional)

Do not freeze



Dry under 45°C

2.1.2 Process

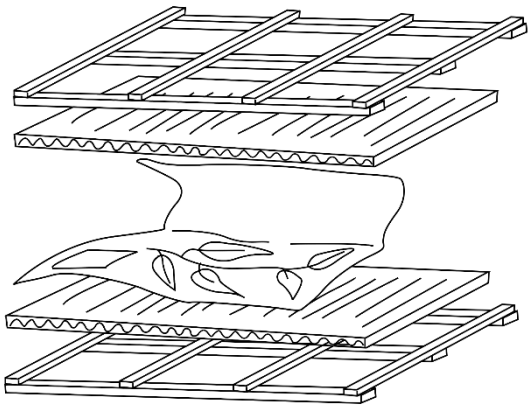
Collections should be pressed and dried as soon as possible to ensure specimen quality is maintained. Plants should be laid out with care as this will determine their appearance when dried. Ensure that disease symptoms are displayed. Excess material should be cut away and discarded.

> Refrigeration can delay degradation, but material will eventually be overrun by fungi, resulting in damage to plant and pathogen, for both morphological and genetic features.

The arrangement of host material is not as important for plant pathology as it is for botanical collections. Often, specimens will be cut into sections to fit in collection envelopes (10x15cm) but ensure that host characters are maintained to aid in species identification.

Ensure full collection details are noted for each pressed specimen and inserted or attached or using a reference number such as collection number or sample submission number.

Newspaper and cardboard are used between specimens. Use several sheets of newspaper to increase absorbency. Sheets of thick cardboard are to assist air movement and drying. Corrugated cardboard is recommended. The newspaper and cardboard are then sandwiched between wooden boards/rigid cardboard to ensure even distribution of weight.



Pressure should be applied to the pressed specimens by some heavy object (bricks or books will suffice).

A purpose-built press can also be used. These generally incorporate a strapping system to keep the bundle together. This makes handling and movement of the collection easier, especially when working in the field. This can then be pressed with added weight once returned to the lab.

The drying process can be expedited using fans, fan heater, air condition vent, drying oven, dehydrator, or desiccant. Changing the newspaper can also speed up drying and also reduces risk of mould growth. Low temperatures are to be used to avoid damaging genetic material. Do not place the press in the sun, it only serves to dry to outermost specimens and can damage them by overheating. Drying time varies from a few days to a few weeks depending on the moisture content of the specimen e.g. brassica material will take longer than other leaf material. The specimen is ready once it no longer feels damp.

Once pressed and dried, specimens should be kept in dry place with consistent temperature and humidity until sending to collections.

Specimens which are thick or damp when collected should have the paper changed every day to prevent mould growth and hasten drying. You can also move these specimens to the drying oven (low temperature, 35 - 45^{o}C) after a day or so once the material is flattened.

2.1.3 Viruses

Virus material is unique. While you can supply it to a herbarium as dried material using the above method, we may also be able to preserve freeze dried samples from fresh material which will remain viable and can revived when reintroduced to the host. Discuss with the collection.

2.2 Dried macro-fungi or bulky material

Bulky specimens include items such as wood sections, branches, fruit, roots, and mushrooms.

Do not freeze

Dry under 45°C

2.2.1 Equipment

- Brown paper bags
- Drying oven/dehydrator/natural light
- Ventilation

2.2.2 Process

For macro fungi and bulky path pathology specimens it is even more important that specimens be dried as soon as possible. Specimens can be stored in the fridge temporarily, but the presence of mould, and especially insects, can cause extensive damage.

These specimens require warm temperatures and adequate air flow to ensure rapid and complete drying of specimens.

Macrofungi are approximately 90% water, you can weigh the specimen before and intermittently while drying to ensure that weight loss has stabilised and therefore all moisture removed.

Specimens are not to be pressed. Lay out specimens on drying racks and dry in a dehydrator or oven on low temperature (<45°C) to ensure morphological characteristics remain true and DNA is not damaged. Alternatively, specimens can be dried using desiccant, although this may need to be changed regularly depending on specimen size.

It is important to ensure that specimens are fully dried. Specimens will be stiff and brittle, if they are still pliable then continue drying. Large specimens may be cut in half or even quarters to improve drying.

Macrofungi change considerably when dried. It is important to take diagnostic photographs when the specimen is first collected. This includes photos of the full habit, cap, gills, base, and if possible a cross section of the specimen showing gill attachment and shape.

2.2.2.1 Spore prints

Spore prints are created by removing the freshly collected mushroom cap and placing it gills down on clean white paper; regular printer paper or filter paper. Cover the cap with a container to eliminate air movement. Leave for several hours. Spore prints can be sealed with artists sealer or hair spray to avoid damage when packing and shipping. Put prints into individual envelopes or plastic sleeves. Ensure that the print itself and the envelope/sleeve is labelled with collection details or unique identifier. Ensure you use a permanent marker and write legibly. Do not use cursive.

2.3 Living cultures

Some herbaria have associated living culture collections. A purified culture of your organism can be stored in a variety of ways including slope media, at ultra-low temperate, freeze dried or as a dried culture (non-viable).

Do not freeze

Every herbarium is different so contact yours ahead of time to see what storage methods are offered if you have specific needs. Furthermore, these preservation techniques are expensive, and far more involved than accessioning dried plant material or dried cultures. We encourage researchers to considered costs of storing specimens generated from research projects and to include these costs when budgeting in project proposals. This aids in supporting collections and also ensures that your material can be accepted and accessioned efficiently.

When sending a culture for accessioning it is good to avoid using antibiotics in your media so that it is easy for the collection to see that your isolate is pure. Not all collections have the resources to perform quality checking on cultures and some are preserved 'as is' meaning if you send a culture that is contaminated it will be put away with that contamination. Generic media, such as PDA, should be used if possible.

The original plant specimen should be retained and provided to the collection with the culture. Refer to 2.1 Dried material - Plant (diseased or host) for preservation method.

2.4 Microscope Slides Microbiology

Microscope slides for diagnostic purposes are often temporary. Even if sealed, these slides are not suitable for long-term storage.

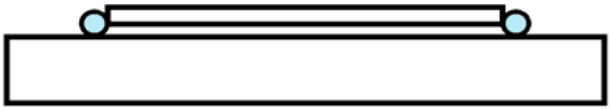
For plant pathology specimens with sufficient material, or living cultures, it may not be necessary to include microscope slides with your specimen unless there is something of particular interest that you would like preserved in this manner.

There are various methods for microbiology slides but for truly permanent slides for voucher material the double coverslip method is recommended.

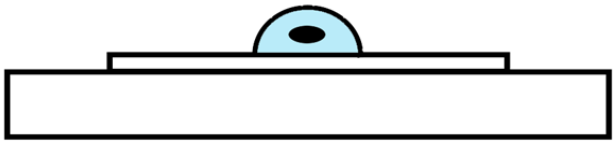
2.4.1 Double cover-slip method

- Slide
- Coverslip 18 x 18
- Coverslip 25 x 25
- Distilled water
- Glycerine
- Nail varnish
- Mounting medium – e.g. Kleermount, in Xylene

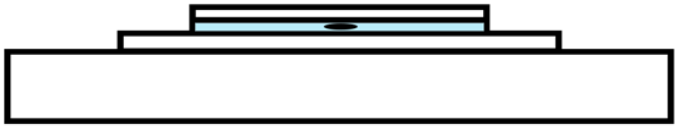
1. Place the 25x25 coverslip on the microscope slide and tack in place with two small drops of distilled water, on either side.



2. Place a larger drop of distilled water in the centre of the cover slip and add your specimen to the drop.



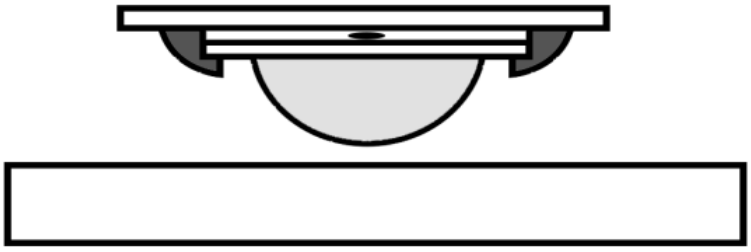
3. The smaller cover slip 18x18 is now used to cover the specimen. The specimen can now be observed. If a desirable arrangement has been achieved and relevant characteristics can be seen.



4. Add a droplet of concentrated glycerine to one or two sides of the small coverslip. The slide is to be stored horizontally in a dustproof container for a few days to allow the water to evaporate and be replaced with the glycerine. After this time, excess water/glycerine can be removed with filter paper/Kimwipes.

5. Seal the mount with a thin ring of clear nail polish. We recommend buying a high-quality brand. Repeat this step after an hour.

6. Once dried, the large cover slip is removed from the slide, a pin can be used to aid in this step. Place a drop of mounting medium in the centre of the small coverslip. The double coverslip ‘sandwich’ is then turned over and placed back on the slide.



7. The medium flattens out, surrounding the edges, this can be aided by a small weight. The slide is stored horizontally until the medium hardens.

2.4.2 Labelling

Labelling slides can be tricky given the small space that is available. Collections will be labelling your slide when accessioned to include collection specific data, like the accession number. When labelling for accessioning use your unique identifier if you have assigned one e.g. personal collector number, otherwise include relevant collection details to identify the slide – species, location, date – as well as the mountant medium used.

These details can be written directly on the slide, a frosted end slide is best in this case. Printed sticker labels, or frosted sticky tape wrapped around the slide to provide a surface for labelling can also be used. Ensure you use a permanent marker and write legibly. Do not use cursive.

2.5 Entomology and acarology material

Here we will present some recommended methods for preparing insect and acarology specimens for sending to collections, either pre- or post-fixing. For further details see *Methods for Collecting, Preserving and Studying Insects and other terrestrial arthropods* by Murray S. Upton and Beth L. Mantle, 2010, The Australian Entomological Society.

Ok to freeze

The easiest way of killing and temporarily preserving insects and mite specimens is to use a freezer until being further processed, preserved or posted to a collection. This serves to kill the organism rapidly and retards the growth of fungi and bacteria pests that could damage the specimen. It is important to avoid repeated thawing and re-freezing as this can damage DNA. The other common way of initially killing and preserving insect specimens is immersion in ethanol, usually at concentrations greater than 70%. Other chemical methods can be used for killing specimens which need to stay dry (moths and butterflies for example). Refer to Upton & Mantle (2010) for further discussion.

2.5.1 Dry preservation of medium and large insects

Medium to large insects are the easiest to process. This is what most people think about when they think of insect specimens – the classic pinned specimen. While it may seem simple at first, the diversity of the insect world has led to several species-specific nuances that make this process more complicated than you might think. However, you do not have to attempt this yourself. Collections have experts trained in this very task to help accession your material.

If you opt for the collection to process your specimens, refer to 4 Posting specimens.

Processing yourself, refer to 2.7 Pinning insect specimens.

2.5.2 Dry preservation of small insects

Small insects cannot be pinned with a regular entomological pin for dry preservation. To minimise damage to the specimen, smaller insects are processed using micro-pins or point mounting onto card, which are in turn staged on a normal entomological pin. Processing such specimens requires a higher level of skill and patience.

If you opt for the collection to process your specimens, refer to 4 Posting specimens.

Processing yourself, refer to 2.7 Pinning insect specimens.

Additional problems arise when sticky mats are used as the collection/surveillance method. Refer to 2.6 Removal of specimens from sticky mats for guidance.

2.5.3 Soft bodied and immature insects

Soft bodied insects such as thrips, aphids, scale, whiteflies, mealybugs and mites can be both collected and killed using 70-95% ethanol[1]. Do not remove mealy bugs or scale insects from the

[1] Isopropanol may be substituted but methylated spirits is not to be used.

leaves or stems; this will damage mouth parts and make identification difficult. Instead, immerse the plant part and insects in ethanol.

Insects can then be sent straight to collections for processing where they may continue to be stored in the ethanol preservative, or slide mounted, or point mounted. Refer to 2.9 Ethanol preserved material.

Alternatively, you may choose to slide mount your specimens yourself. This is a lengthy process and success will depend on your level of experience and expertise, it may be best left to the collection to process in this way. Please refer to 2.8 **Error! Reference source not found.**Microscope Slides - Entomology & Acarology.

2.6 Removal of specimens from sticky mats

Sticky mats are a popular choice for insect monitoring and trapping. Unfortunately, the method makes specimens retrieval difficult. Here we detail a method for removal of specimens.

Ok to freeze

2.6.1 Equipment

- Oomph* – or similar product (citrus-oil based cleaner, kerosene)
- Fine tweezers, probe, paintbrush
- Pipette
- Ethanol (70% to 100%) *
- Detergent

*WARNING: consult the MSDS for safety advice

2.6.2 Method

A small drop of Oomph/cleaner applied to the insect should dissolve the polybutene 'tanglefoot' enough to allow the removal of the insect with fine forceps after 10-15mins of soaking.

Using either your paintbrush or a probe, delicately remove the specimen(s) to a cavity block of kerosene or oomph for further dissolution of sticky trap coating for up to 20 minutes.

Using a pipette, carefully decant all kerosene or oomph from specimen(s) in cavity block. Be sure not to decant the specimen(s) and try not to touch the specimen(s) with the pipette tip.

Pipette 1-2mls of 50/50 detergent-water mixture into cavity block using pipette. If the specimen floats there is still too much kerosene or oomph present. Fill a new cavity block with the water-detergent mix and transfer the specimen. Leave specimen(s) in cavity block of detergent-water for 10 minutes.

Decant the water-detergent mix using a pipette and then soak the specimen in plain water for 5 minutes.

The insect can then be transferred to 70% alcohol, or if retaining for DNA analysis dehydrated using increasing concentrations of ethanol until 100% is reached, leaving the specimens for 10 minutes at each concentration (70, 80, 90, 100%).

Label the vial with collection information. Include the alcohol concentration.

2.6.2.1 Limits

Some organisms, particularly those that are small (<2mm), those with long skinny appendages (some flies) or that are covered in scales (moths) are easily removed from sticky traps, however they have a tendency to break, leaving large parts of the insect behind.

2.7 Pinning insect specimens

2.7.1 Medium and large insects

Dry under 35°c

Specimens must be pinned shortly after killing, while pliable, as legs, wings and antennae need to be moved into a position that facilitates examination and minimises risk of damage. If your specimens become fully dried, they can be rehydrated, or 'relaxed', to allow positioning of appendages. This is performed in a relaxation or humid chamber. Specimens can take hours to days to relax, depending on age and condition. For more details refer to *Methods for Collecting, Preserving and Studying Insects and other terrestrial arthropods* by Murray S. Upton and Beth L. Mantle, 2010, The Australian Entomological Society.

! Relaxing specimens destroys DNA and potentially other chemical signatures. If necessary, a leg can be removed before relaxing, this can be stored in a gel capsule and included on the pin to allow for DNA extraction in the future.

Medium and large insects can be pinned using a standard size 2, 3 or 5 entomology pin depending on the specimen size[2]. There are specific requirements for how certain species are pinned, such as the location of the pin through the body, and winged insects need additional care and skill as wings must be spread and supported during pinning and drying. For details on methods for arranging specimens and mounting onto the pin, we recommend consulting *Methods for Collecting, Preserving and Studying Insects and other terrestrial arthropods* by Murray S. Upton and Beth L. Mantle, 2010, The Australian Entomological Society.

The physical state of the specimens as well as it's size must be considered when choosing a method. For example, specimens stored in ethanol may require card-point mounting as the ethanol can make them brittle and re-hydrating would destroy DNA.

[2] https://www.entosupplies.com.au/pins-insect-micro-points/

2.7.2 Small insects

Small or thin insects (<10mm) will be damaged if pinned with a standard entomology macropin. These specimens can be pinned with a fine headless micropin (minuten), to a stage that is then pinned onto a standard macropin, or they can be glued to the tip of a card point that is pinned on a standard macropin.

There are specific requirements for how certain species are orientated on the stage/point and where the pin is mounted through – dorsal, ventral obliquely. For details on methods for arranging specimens and mounting onto the pin, we recommend consulting Methods for Collecting, Preserving and Studying Insects and other terrestrial arthropods by Murray S. Upton and Beth L. Mantle, 2010, The Australian Entomological Society.

2.7.3 Labelling

Labels are mounted onto the specimen pin; if specimens are staged on a micropin or card point the labels are on the main pin. Labels are to be applied to all specimens, even when there are specimens from a series with the same collection details. Labels are to face upwards and, where multiple labels are needed, placed at spaced intervals to facilitate reading without disturbing the specimen. The collection data label should always be the top label. Labels should be made as small as possible while still remaining clearly legible. Extensive collection details can be split up onto multiple labels.

2.7.4 Drying

In drier climates, natural drying is possible however, artificial drying of specimens can be beneficial to speed up the drying process. Do not use temperatures above 35°C as this can affect colouration and cause curling of delicate features, like wings.

Large specimens may require up to 3 weeks to dry fully under natural conditions. Ensure specimens are stored in a pest-free space while drying.

2.8 Microscope Slides - Entomology & Acarology

Many insects and their allies are preferentially diagnosed and preserved via microscope slides. Microscope slides for diagnostic purposes are often temporary. Even if sealed, these slides are not suitable for long-term storage, they inevitably dry and crack, often within months, even if sealed, frequently destroying the mounted specimen.

Long-term slide mounting has lasting benefits for many taxa. Generally, these mounts take longer to prepare and may lack the clarity of water-based mountants, however the hydrophobic basis of these mountants ensures their long-term viability (years/centuries). Long term mounts can also be

remounted and a number of new formulations/processes refining non-water miscible slide mountants have greatly improved their clarity.

Note that there are many slide mounting techniques and media, often developed by specialists, for specific taxa. Here we offer a general method.

Slide mounting specimens using Canada Balsam produces a slide that is proven to last for hundreds of years and remaining stable and in good condition.

2.8.1 Equipment and reagents

- slide – use good quality slide Menzel-Gläser 76 x 26 mm with ground edges
- coverslip[3]
- glass Pasteur pipettes and bulbs
- glass cavity blocks/small petri dishes
- forceps
- metal probes
- micro spoons
- kimwipes
- worksheet (as attached)
- 70%, 80%, 90% & 100% ethanol *
- KOH 10% *
- Stain – acid fuchsin *
- Clove oil *
- Canada balsam *
- Ammonia solution – strong 28% *
- Hydrogen peroxide – 6%w/v *
- Oomph *

***WARNING**: consult the MSDS for safety advice

2.8.2 Method

Clear the specimen – Soaking of body using 10% KOH – place the specimens in a cavity dish with a small amount of 10% KOH and leave for 20 minutes, or longer, for the body contents of the specimen to clear. Constant monitoring is necessary as the time may vary for different types of insects. The body of the specimen can be pierced/cut to allow the KOH to enter the body cavity quickly and allow the gentle removal of digested body contents. Be sure to pierce the body in a position that will not destroy diagnostic features e.g. small incision in the side of scale insects or pierce hole in abdomen of aphid. The specimen can be gently prodded to remove body contents and

[3] Size 0/12mm round for most specimens. Can use larger and heavier for bigger specimens)Size 1 is preferred for mites and for other insects with minute structures. Oil immersion objectives are usually optimised for this thickness. You can check the coding on the objective to make sure you match the coverslip size to the objective.

any air bubbles from inside the specimen. Soaking may take days depending on the size and colour of the specimen.

If the specimen needs to be processed quickly it can be placed in a small amount of 10% KOH in a collection tube and placed in a 60°C water-bath to quicken the process, alternatively the specimen can be placed in a covered glass cavity block and placed on a slide warmer at 75°C. This may take from 20 minutes to 40 minutes or a few hours depending on the size and colouration of the specimen.

WARNING: Be sure to point the opening of the test tube away from you in case of fumes and splash and work in a well-ventilated area.

For very dark to black specimens, such as some whiteflies and thrips, the clearing process can be promoted by removing the specimen to small but equal volumes of strong ammonia and new hydrogen peroxide (both stored in fridge to keep them fresh) instead of KOH. This can be mixed in a small petri dish then covered with a larger petri dish (strong smell).

WARNING: Care should be taken with strong ammonia as it can be harmful.
WARNING: Check frequently as the process can be very quick (sometimes only seconds).

You should be able to see the structures on the dorsal and ventral side by looking through the specimen with still a good level of colouration.

De Wax the specimen – de waxing can be done during the clearing stage as the KOH will slightly soften and dissolve the wax. De wax by gently prising the wax cover from the soft body of the insect. If the wax is not easy to remove place the specimen in Oomph for up to 1 hour. Refer to removing insects from sticky traps for how to process specimens in Oomph.

Dehydrate the specimen - Once the specimen has been cleared wash twice in 70% ETOH. Dehydrate the specimen from 70% ETOH to 100% ETOH in no greater than 10% increments. Wash twice in 100% ETOH. The specimen should be in each concentration for at least 15 minutes for each step.

WARNING: the gradual dehydration ensures that the specimen does not distort or crumple thus making it hard to arrange on the slide.

Stain the specimen - Whiteflies, scale, mites and some very clear aphids may need to be stained. Thrips, Psylloidea and aphids generally do not need to be stained.
Stain the specimen by placing it in a small amount of Acid Fuchsin using a micro spoon. Leave for up to 5 minutes and maybe 30 minutes depending on quality of the stain. Remove using a micro spoon and place in 100% ethanol to wash off the excess stain in preparation for the next step.

Clove oil - Place in clove oil in preparation for slide mounting. Leave for at least 30 minutes. The clove oil is used to clear the specimen in preparation for transferring to Canada balsam.

Slide-mount the specimen – Prepare coverslip and slide by washing in 100% ETOH and wiping the slide with a kimwipe. Carefully remove the specimen from the clove oil using a microspoon and place on the cover slip/slide. Gently manipulate the specimen so that it is centrally located on the cover slip/slide and all body parts are positioned for ease of identification.

- If preparation is done on the cover slip the specimen will need to be ventral side up and head away from you.
- If preparation is done on the slide the specimen will need to be dorsal side up and head closest to you.

Using a kimwipe with the edge twisted to a point, or filter paper triangles, remove the excess clove oil so that only a very fine film is left on the specimen.
Place a drop of Canada Balsam on the specimen by dipping the probe into the bottle of Canada Balsam and letting the first few drops return to the bottle then take the next drop onto the specimen on the slide/coverslip. Do a final adjustment of the position of the specimen then spread the Canada Balsam towards the edge of the coverslip using a needle or micro spoon. The coverslip/slide can then be gently lowered onto the specimen.

The size of the coverslip is dictated by the size of the specimen being mounted. It is preferable to use the smallest and thinnest coverslip feasible to minimize distortion of the specimen.

Label the slide with collection details or collection number.

Dry the mounted specimen - Place the completed slide on a tray then in the slide drying oven (60^{o}C) for at least 4 weeks to ensure the Canada Balsam dries. The Canada Balsam may shrink and leave a gap under the slide. This can be refilled with a drop of Canada Balsam placed beside the coverslip so that it is sucks into the gap. Place back in the oven for further drying.

To de-mount a specimen: Soak the slide in 100% alcohol in a petri dish, gently removing the coverslip and slide, ensuring the old Canada balsam is soaked off.

2.9 Ethanol preserved material

Some specimens are best suited to alcohol ethanol storage, for long-term, convenient transport to collections, and to ensure the organism is dead. Soft bodied insects, immature stages, mites and other arachnids must be stored in ethanol to prevent them shrinking and collapsing in on themselves. Moths and butterflies (Order Lepidoptera) should **not** be stored in ethanol as diagnostic features associated with scales on the wings and body will be lost. These insects are best pinned directly for transport. See Upton & Mantle (2010) for further details on requirements for these insects.

2.9.1 Equipment & reagents

- Suitable sized leakproof tube – screw top or clip-lock tube.
- 100% ethanol – when collecting primarily for DNA extraction
- 80% ethanol – when morphology is priority, but DNA is still preserved well when chilled.
- Fine tipped forceps
- Mail declaration

2.9.2 Method

Pre-fill tubes with ethanol. Using fine tipped forceps, pipette or paint brush, transfer specimen into pre-filled tube. Specimen details (unique identifier or collection details) can be written in pencil on paper, cut to size, and placed in the tube with the specimen. This is best practice as pen, even alcohol proof pen, may dissolve if the tube leaks.

Labels can also be written on paper-based tape and wrapped around the tube. This also allows for more information to be included when using smaller tubes.

Ethanol may need to be changed if large specimens or multiple specimens are stored in a single tube; this is to ensure that the specimen is properly dehydrated by the ethanol. Unless posting the specimens keep the vial full of ethanol.

See 4 Posting specimens for details on posting alcohol stored material.

2.10 Nematodes

For a comprehensive reference on nematode collection and preservation we recommend the following resource: Guidelines for the management of nematode collections used for the production and maintenance of reference material, EPPO Bulletin. 2021. 51: 507–548. https://onlinelibrary.wiley.com/doi/10.1111/epp.12798

Refer to appendix 10 and 11 for details on slide mounted preservation.

Nematodes can also be stored in alcohol. Refer to 2.9 Ethanol preserved material.

3 Collection data and peripheral materials

3.1 Collection data

> ! There are many ways to collect and store data. It is best to contact the collection ahead of time and establish in what format they prefer data be provided.

Collection data is any data related to the specimen at the point of collection. Information to consider when collecting a specimen includes:

Collector's name: *[mandatory]* the name(s) of the person/people who collected the specimen. For group expeditions such as student bodies or enthusiasts, you may use a group name e.g. Sydney fungal studies group 2023 foray.

Collector's number: *[optional]* A unique identifier given by the collector for their personal records.

Collection method: *[optional]* Trap type, attractant (lure, light etc.), reared

Date of collection: *[mandatory]*. Date format must be yyyy/mm/dd (e.g. 2023/08/24).

Species identification: *[optional]* Both scientific name and common name. If you are unsure of the identity of the pest or disease it is still helpful to suggest a name, or at least a genus.

Date of identification: *[optional]*. Date format must be yyyy/mm/dd (e.g. 2023/08/24)

Host identification: [optional] see above.

Locality: *[mandatory]* A description of the precise collection locality and latitude and longitude coordinates. A GPS location alone is not sufficient as numbers often suffer from transcription errors.

Locality should include enough information to enable the approximate place to be revisited. This precise location is usually made up of a description that includes distance and/or direction from a town or a well-known locality. It should be meaningful to someone not familiar with the local area. DO NOT use specific farm/property names or people as reference. We have records in the collection that say: "in the backyard of XX's house". This will be meaningless in the future.

Precise location should not include information that is better suited under "Habitat".

Geocode: *[mandatory]* Copy the GPS reading obtained in the field in DECIMAL DEGREES. Smart phones can access geocode data via the map app even when no cell phone signal is available.

Altitude: *[optional]*

Habitat: *[mandatory]* Details on the surrounding area where the specimen was collected, e.g. roadside vegetation, glasshouse, forestry nursery.

Pest/disease incidence: *[mandatory]* A comment on the frequency of pest/disease at the collection site. You may use terms such as "widespread" or "dispersed", or you can give estimated percentages of affected plants.

Other notes: *[mandatory]* Any other relevant information, for example, a reference to a photographic image or material in spirit, or additional trophic relationships.

3.2 Images

Images can be supplied to collections in digital form, either by file transfer or on a storage device provided with the specimens. Images should be supplied at the highest resolution/file size available and in RAW or TIFF format, if available. The collection can then make different image sizes for different uses as needed.

If providing hard copies, we suggest printing these on photo paper as regular printer paper is prone to damage and ink will fade with time.

3.3 DNA and DNA related data

DNA can be included with a specimen and can be sent in solution or as a dried pellet. Include details on extraction method used and DNA quantity and quality.

DNA data can be provided as the unique identifier from an online repository e.g. GenBank accession number, or a digital copy of sequence data in .txt file format.

You may also include analyses such as alignments, in .txt file, or phylogenetic trees, in .pdf format.

3.4 Notes and other documents

These can be shared digitally or as hard copies. Ensure that all items, whether digital or hard copies, are clearly labelled and include the specimen details that the items relate to.

Some examples of documents you may want to include with your specimen are:

- Diagnostic reports
- Surveillance reports
- Morphological descriptions
- Correspondence related to identifications or biosecurity status

4 Posting specimens

4.1 Insects

4.1.1 Unpinned material

Certain species of fresh specimens for pinning can be sent to collections preserved in ethanol, see 2.9 Ethanol preserved material In this instance your specimen will be removed from the ethanol for processing rather than stored this way. Moths and butterflies (Order Lepidoptera) should not be stored in ethanol as diagnostic features associated with scales on the wings and body will be lost. These insects are best pinned directly for transport. See Upton & Mantle (2010) for further details on requirements for these insects.

Specimens can also be sent in containers. It is important to use a suitable sized container to avoid movement of the specimen during transit. Specimens can be lightly wrapped with kitchen paper or loosely packed with tissues or cotton wool to limit movement. Do not place insects together as their appendages can become entangled and break.

For medium to large insects, it is recommended that extra absorbent material be added to the container, or ventilation holes be added, to avoid moisture build up and mould growth.

Pack your container in a zip-lock bag with additional absorbent material and pack into a box with loosely packed packing material for shock absorption during transit.

4.1.2 Pinned material

Pinned material is the most fragile to post. Specimens are easily damaged or destroyed because they have been packed improperly.

For posting, use a strong wood or cardboard pinning box, with a layer of cork or pinning foam well fastened to the base.

Specimens are pinned into this foam; larger insects should be cross pinned with four pins that will hold the body in place and prevent the specimen from spinning around on the pin. The pins are used on a 45^{o} angle running above and below the body on each side. The lower pins are not needed if the insect is resting against the foam/cork base.

The box should be seal in cling wrap or a plastic bag to prevent dust and moisture. Pack this into a good quality carboard box that is at least 15cm larger than your pinning box. Loosely pack the package with packing material. Do not pack the specimen too tightly or the shock absorbing effect of the packing material is reduced.

4.2 Plant material

Do not send your stack of newspaper and specimens to the collection; specimens may fall out and be separated from their collection information.

Pack your specimens into envelopes with collection data or your unique identifier clearly labelled on the envelope or included inside. Collection data should be provided in hardcopy with the specimen, even if you have provided it electronically. It is good practice to also include a cover letter explaining the contents of your package or a copy of email correspondence.

Pack everything into a rigid mailer, or sandwiched between cardboard, or in a box.

4.3 Macro fungi

Specimens can be packed with paper towel in rigid containers to prevent damage, then into a larger postage box with packing material. Include collection details or collection reference number within/on the specimen container with a hard copy of collection details spreadsheet/copy of emailed details included in the package. Avoid sending specimens to collections with sparse details e.g. only citing your collection numbers with details 'to follow'.

For posting fresh material, specimens are to be sent in a rigid container with paper towels to hold the specimen in place and absorb excess moisture. Do not add moisture. Fungi are not like plants and excess moisture will degrade the specimen. Store them in the fridge until posting and send with ice packs, you can use an insulated carry bag like a lunch bag to help maintain temperature. Post using express post.

4.4 Living culture

When sending cultures to collections it is important to ensure that they are pure cultures, this means growing them on general, antibiotic free media such as PDA. Send cultures that have been freshly grown. Make sure you label the culture and include the date it was subcultured. Use two layers of parafilm to seal the plate and place within a rigid container with protective material to avoid cracking or damage to plates.

4.5 Microscope slides

Slides are obviously fragile, and care should be taken when packing them. Ensure slides are clearly labelled with collection details, or collection number and hardcopy of details included in the package.

Slides can be placed in purpose-made slide mil, these come in carboard or plastic, or slides can be sandwich between cardboard and taped.

4.6 Specimens in ethanol

In this instance it is important to be aware of the shipping requirements for sending ethanol in the mail. Specimens preserved in ethanol, or solutions of ethanol, may be sent by courier and Australia Post. These instructions are for packaging and sending to meet the International Air Transport

Association Regulations Special Provision A180. They are based on the information at the CASA website: https://www.casa.gov.au/standard-page/special-provision-a180

Specimens should be in rigid, leak proof, containers with no more than 30ml of ethanol or ethanol solution in each vial. 100% ethanol is recommended as this ensures dehydration of the specimen.

Each container must be in a separate heat-sealed bag, or several vials can be in same bag with a heat-seal separating each one. Place all sealed bags into a single plastic bag (or multiple bags if required) containing absorbent material e.g. absorbent cotton wool or paper towel and heat seal.

Place the finished bag or bags into a sturdy box containing cushioning material. Ensure that the total quantity of ethanol or ethanol solution contained in the outermost package does not exceed 1L.

Mark the completed package with: "*Scientific research specimens, not restricted, Special Provision A180 applies*" and include arrow labels to indicate "this way up" orientation.

Mark the Consignment note or Waybill with the words: "*Scientific research specimens, not restricted Special Provision A180 applies*"

Ensure full collection data accompanies specimens and it is good practice to include a cover letter, or copy of correspondence, explaining the package contents.

4.7 Customs requirements

We recommend lodging specimens with collections from the state they were collected but this isn't always possible. Should you need to send specimens to a collection interstate you must check that the material you are sending is not prohibited matter, even if the specimens are non-viable you may require a permit or Chief Plant Protection Officer permission to transfer such specimens. The collection should be able to assist you with this.

4.8 Collection data

It is good practice to include the collection details in hardcopy in the parcel, even if you have emailed them through ahead of time or plan to provide them electronically. This may be included with each specimen or as a separate document with reference numbers, where specimens are labelled with these unique reference numbers.

If you are accessioning large numbers of specimens the collection may have the capacity to import collection data from bulk files, which can be emailed ahead of time. If this is done it is important to include a copy of the email or a cover letter explaining the parcel contents and that the collection data has already been supplied.

5 Budget for vouchering

While accessioning into collections has traditionally been free of charge, the growing burden on collection resources is making this an unsustainable practice.

Where specimens are unique and of significance to national biosecurity - first reports of a pest or disease either in the country, state or for that location or host – accessioning is generally covered by the collection as these specimens are strengthening our biosecurity framework.

It is important to consider the time and costs involved that are being passed on to the collection when submitting specimens. If large collections are anticipated for a project or you expect the collection to fully processes specimens (pinning, slide mounting), we encourage you to consider these costs and budget for the work. Contact your jurisdictions collection to discuss further.

6 Frequently asked questions

6.1 What happens to my specimens after they are submitted?

When they arrive, your specimens will be examined to ensure they have been processed correctly and then they are stored at -20^{o}C to ensure any insect pests have been killed. All parts of the collection are freeze treated including paper materials.

Specimens are placed in archival quality storage, such as packets, boxes or insect trays. The photographs and notes are kept with the specimen or a separate filing system, if too large.

Each of your specimens will be given a unique accession number, this is used when referencing the collection in publications. Your specimen collection details and notes will be transcribed or imported into the database and a printed label will be included with the specimen. Specimens are then filed in the collection.

There may be a backlog of specimens awaiting processing. If you need your specimen accessioned urgently e.g. an upcoming publication, please advise staff at the point of submission.

6.2 What if I don’t have the species identified?

Collections do not always offer an identification service, but unidentified material is not entirely useless. Your specimens may eventually be examined by experts working on the taxonomy of a particular group or pests and diseases from the host you have collected.

Having the physical specimen means we can find out more on the species identification later, so unidentified material still has value.

7 Further Reading

Murray S. Upton and Beth L. Mantle, 2010 *Methods for Collecting, Preserving and Studying Insects and other terrestrial arthropods* The Australian Entomological Society.

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Australian Government
Department of Agriculture, Fisheries and Forestry

NATIONAL PLANT BIOSECURITY DIAGNOSTIC NETWORK

Plant Health AUSTRALIA

NSW GOVERNMENT Department of Primary Industries