Sending Material to the Diagnostic and Advisory Service

Information is provided here on how samples and cultures should be collected, prepared and sent to the Diagnostic and Advisory Service. Standard sample submission forms, which should be completed and sent with the samples, are available on the Diagnostic and Advisory Service web pages.

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If possible, please contact the Diagnostic and Advisory Service (diagnostic.service@cabi.org) before sending samples and cultures. This will allow Service staff to provide you with advice before you send the sample, and also give them an opportunity to ask for any additional information which would be required for an accurate diagnosis. You will be provided with an enquiry reference number, UK Import permit ('Letter of Authority') and any other relevant documentation.

1. Collecting, preparing and packing (plant material, nematodes and soil)

Collecting and preparing

- Collect plant material that shows early, middle and late stages of the problem if possible. Do not collect and send tissues that are completely dead or completely necrotic. Usually, samples showing the early stages of the disease are the most promising for making isolations
- Fresh samples, collected the same day, offer the best opportunity to isolate and identify pests and diseases, especially bacteria. However, please pack and send fresh samples to the UK only if you are able to use a courier service that can guarantee delivery within 1-2 days
- Otherwise, dry samples prior to dispatch as this prevents the growth of unwanted saprobes. Most fungi, viruses and phytoplasmas tolerate the drying process well. The samples need to be dried for several days depending upon their size and water content
- It is recommended that leaves are pressed during drying to speed the process and avoid curling. Please do not dry samples in an oven. Fresh, damp material should not be sent as it rapidly deteriorates in transit
- Avoid over-handling of samples, especially where a virus or phytoplasma problem is suspected, as natural chemicals on your fingers can interfere with our testing procedure
- Never wrap herbaceous plant material in plastic unless nematodes are suspected to be present in the material
- For roots: shake off excess soil and wrap each sample individually in newspaper
- For stems and leaves: include healthy and diseased samples for comparison, but wrap them separately in paper. Avoid thin absorbent paper like tissues as these disintegrate and are difficult to remove
- For soil: remember to double bag soil samples, with labels included with the soil (these should be placed between the two bags). Write labels in pencil as this is more durable than ink.
- Delicate specimens are best stuck down onto stiff card before being sent
- Senders are advised to retain a duplicate of each sample, as material submitted for identification is not normally returned by the Diagnostic and Advisory Service

Packing

- Each individual sample should be placed inside a separate envelope and labelled with the sender's own reference number for the sample
- If sending killed and fixed nematodes in glass sample bottles, carefully wrap each bottle in a plastic bag and ensure they do not leak. Parafilm provides a good watertight seal
- Pack all samples carefully to avoid damage in a strong, crush-proof container.
- Add additional paper, cardboard or polystyrene granules to the container to protect the samples and prevent them moving during transit.
- Samples should be protected from wide temperature fluctuations
- Samples of plant material rapidly deteriorate and decay in transit. They should therefore be sent by airmail where possible
- If sending mounted slides, place them in a slide carrier or slide box

2. Preparing and packing cultures (fungi and bacteria)

Fungal and bacterial cultures may also be submitted for identification. Identifications are usually undertaken by CABI Microbial Services staff based on morphological examination and/or molecular analysis. A general guide to preparation and packing of cultures for submission is provided below. However, before sending cultures you should also refer to the detailed information available at the Microbial Services website.

Preparing

- Send fresh isolates as soon as growth has been established in subcultures. Ideally they should not be more than 3 weeks old on arrival.
- Pure cultures must be submitted for identification. Cultural mutants or non-sporulating strains cannot be identified. Non-sporulating cultures can sometimes be induced to sporulate by placing under near-ultraviolet light (see **section 7** below).
- Cultures should be grown on firm agar media. Suitable agars for fungi include Potato Carrot Agar, Potato Dextrose Agar (25% strength) or Oat Agar. Malt Extract Agar is recommended for yeasts, and Nutrient Agar or Tryticase Soy Agar for bacteria.
- We recommend that you keep a subculture of each isolate submitted for identification in case there is a need to resubmit material. This will also enable you to examine your cultures after we have sent you our report. Cultures are not generally retained at CABI or returned to you after identification.
- Send cultures in either: 30ml Universal containers (glass or plastic); small McCartney bottles (glass or plastic); small glass test tubes (100 x 15 mm); or as freeze-dried ampoules.
- Petri dishes are not recommended for sending cultures from overseas as these are easily damaged or contaminated in transit.
- If possible use clear containers to permit clear observation of fungal and bacterial growth. Avoid sending cultures in opaque containers.
- Check that bottle caps are not over-tightened. If the seal is too tight the fungi and bacteria are likely to die.
- If using test tubes, they should be sterilized by flaming the neck and fitted with a sterile cotton-wool plug. Otherwise use tubes with screw tops.
- Cultures must be free of contaminants and mites. As mites introduce rapidly-growing contaminants that are easily spread, any cultures containing mites will be destroyed immediately on arrival at CABI. Check for mite contamination and, if mite-free, seal the cultures. The cigarette paper sealing technique is a useful way of protecting cultures from mite contamination.
- Ensure all tubes and containers are labelled with a reference number. Use an adhesive label (write using a pencil) or write directly on the tube with waterproof ink. Avoid grease pencil, as this easily nubs off.

Packing

- When packing and sending living microorganisms to other countries, international regulations for packing and postage must be adhered to. For further details see the Microbial Services website.
- Information on the regulations covering postal packages can also be obtained from IATA (International Air Transport Association) or viewed at http://www.eccosite.org/transport.html
- United Nations approved packaging should be used according to the hazard group of organism being transferred.
- These regulations do not apply when sending dead, dried material. However, sturdy packaging is necessary to avoid damage to the material in transit. Our experience has shown that a padded

envelope alone does not give sufficient protection. If using such an envelope the specimen(s) should be placed inside a folded sheet of stiff cardboard as well.

- Each country has its own national regulations covering the package and transport of microorganisms in domestic mail.

3. Documentation to include with packages

- All of the documents specified below should be obtained, completed as necessary and included with each package submitted to the Diagnostic and Advisory Service:
  - Diagnostic and Advisory Service sample submission sheet
  - Documentation required to clear customs in your own country
  - Relevant import permits ('Letters of Authority') to clear customs in the UK
  - Other documentation of relevance to the samples or cultures
- The Diagnostic and Advisory Service enquiry reference no. (if you already have one) should be marked on each document AND on the outside of the package(s)
- You are advised to retain a copy of all documentation sent with your package as well as mailing documents
- Failure to include the necessary customs documents may result in the package and its contents being destroyed by UK Customs
- For UK import permits ('Letters of Authority') - complete Section 1 ('Name and address of Consignor/Plant Protection Organisation') and Section 13 ('Endorsement') of the following letters of Authority and include with your package(s). If a phytosanitary certificate for the package has been issued by the country of origin, please also complete Section 6 ('Plant Passport or Phytosanitary Certificate no.').

4. Where to send samples

Packages should be addressed to:

Diagnostic and Advisory Service
CABI UK
Bakeham Lane
Egham
Surrey TW20 9TY
United Kingdom

Packages should be clearly marked with the following:

- Perishable Biological Material
- Keep material cool but do not refrigerate

5. When to send samples

- Try to avoid sending samples over a weekend or at other times when CAB I is closed.

6. Dispatch checklist

- Have you completed the sample submission form with all relevant information and included a printed copy with your samples?
- Have you printed out, completed and included the correct UK import licence(s) ('Letter of Authority')?
- Have you completed and included all the necessary forms for dispatching the samples from your country, including local permits?
- Have you contacted the Diagnostic and Advisory Service to let them know that you are planning to send samples/cultures and when they are due to arrive?
- Have you packaged the samples in appropriate materials that will protect the samples during postage and conform to the regulations covering postal packages?
- Have you checked the dates when CABI UK will be closed? If in any doubt about the above instructions and before sending samples please contact the Diagnostic and Advisory Service for advice.
- Our obligation to the Convention on Biological Diversity (the Rio Convention) means that CABI must ask you if any of the material you send for identification was collected after December 1993. If that is the case then you need to inform us if prior consent was received to collect it. This should have been obtained from the land owner and/or the relevant national authority.

7. Use of black light to induce culture sporulation

- Some fungi require irradiation by near ultraviolet light to induce sporulation in culture. Near ultraviolet light is often referred to as 'black light' (wavelength 300-380nm). Although black light may affect such factors as pigmentation, spore morphology and the gross morphology of the colony, these effects are not sufficient to interfere with identification
- In our laboratories the black light unit consists of three 1.22m fluorescent tubes fixed beneath a bench top and connected to a timing mechanism. A near ultraviolet light tube (Phillips TL 40 W/08) is placed between two cool white tubes (Phillips MCFE 40 W/33) and the cultures are supported on a shelf 32cm below the light source. A wooden shield extending 15cm below the bench top, is fitted around the unit to prevent light rays from directly striking a person. An alternating cycle of 12 h UV and 12 h darkness should be established as some fungi require a dark period in order to sporulate
- The fungi are grown in plastic Petri dishes or plastic universal bottles. American 'Pyrex' Petri dishes can also be used. Glass however, is not suitable as it does not allow adequate transmission of near ultraviolet light
- Many fungi sporulate most successfully on nutritionally weak media such as PCA (Potato Carrot Agar), OA (Oat Agar) or TWA (Tap Water Agar). Unless recommended for particular genera, sugar rich media should be avoided as this promotes excessive amounts of mycelium. The dishes are sealed with electrical tape or sticky tape to prevent rapid drying of the culture and infestation by mites. Cultures should be checked regularly for signs of sporulation
- Irradiation should start 3-4 days after inoculation. Except in the case of thermophilic or psychrophilic fungi, the optimum temperature range for growth under black light is usually between 21-28°C. Temperatures in excess of 30°C should be avoided as the effects of black light can be lost at high temperatures