

# Sample collection and submission to the CFIA Rabies Laboratory

This document provides information about the collection of a rabies suspect sample and how to submit it to the CFIA rabies laboratory for diagnosis, including:

- the [submitter's responsibilities](#), in particular, with regard to the [Transportation of Dangerous Goods](#)
- [how to collect the sample](#)
- [how to submit the sample](#) to the CFIA rabies laboratory, including packaging, shipping and documentation
- a list of [DO's and DON'Ts](#) associated with these activities
- and a [final review](#) of the complete process

To determine if a sample should be submitted for rabies testing, visit the [Rabies Guidance page of the CVMA website](#) and consult the guidance document, "General information and submission guidelines."

## Submitter's responsibilities – Transportation of Dangerous Goods

The Submitter is responsible for complying with the requirements of the [Transportation of Dangerous Goods Act](#) and [Regulations](#) pertaining to the packaging and shipping of infectious substances. These requirements include training and certification.

Proper identification, handling, packaging and shipping ensures:

- biological and physical hazards to the public, transporters and laboratory staff are minimized
- an increase in the probability of prompt delivery to the CFIA rabies laboratory
- maintenance of sample quality, which leads to timely and accurate test results.

## Collection of samples

### Information required for rabies sample submission form

Certain information about the sample should be confirmed at the time of sample collection, including:

- the species of the animal

- any information regarding the nature of the exposure.
  - if you are considering collecting samples from a human suspected of rabies, phone the Ottawa Animal Health Laboratory at (343) 212-0340 for instructions.
- location where the sample was found, using geo-referenced coordinates, that is, longitude and latitude in the decimal degree format.
  - this location should correspond the site of the exposure event, not the location of an intermediary party (for example, a veterinary clinic) from which the sample was retrieved.
- the condition of the carcass, such as damage to the head, evident decomposition, insect infestation, as well as the presence of physical hazards such as quills, bullets or darts, should be noted
  - only fresh or frozen samples can be submitted for rabies testing. Formalin-fixed samples cannot be submitted.

Up to four email addresses may be indicated on the submission form for transmission of test results (e.g. provincial coordinator, intermediary party/veterinary clinic, etc.).

For more information, visit the [Rabies Guidance page of the CVMA website](#) and consult the guidance document, “Completing the electronic submission form.”

## What to submit

The sample that should be submitted to the laboratory will depend on the size of the suspect animal.

For most animals:

- submit the entire head
- include the proximal cervical spinal cord if there has been trauma to the head.
- If the body of a pet needs to be retained, then extract and submit the entire brain (no part of any animal submitted for rabies testing can be returned to the affected party).

For small animals (<500g, e.g. bats):

- submit the entire carcass to permit confirmation of the species
  - this is particularly important for bat submissions, which can be challenging to speciate by morphology

For large animals (>100 kg, e.g. all livestock)

- submit the entire brain (extracted from the cranium - submission of the entire head will result in delays in testing) **and** proximal cervical spinal cord

- if it is not possible to submit the entire brain, collect portions of brain tissue bilaterally from the cerebellum, hippocampus and brainstem, as well as some proximal cervical spinal cord

## Disarticulation of the head

Collection of a head begins with the disarticulation of the head from the spinal column of a humanely euthanized animal.

- Remove the head at the base of the skull using an axe, knife or handsaw. Include some proximal cervical spinal cord when indicated.
- Always leave the head intact without removing skin, ears, jaw or snout.
- Disinfect the collection site and tools used in the process.
- Dispose of the carcass in compliance with all applicable local legislation.

## Extraction of a brain

For livestock and other large animals (>100 kg), the brain should be removed from the cranium for submission. Collecting a brain is straightforward but can be difficult due to bone thickness.

- Remove head at the base of the skull with an axe, knife or handsaw
- Secure the head with the dorsal aspect upright
- Skin the head from neck to snout across the cranium
- Make 3 cuts with a chisel or handsaw to penetrate the full depth of the cranium and intersect above the eyes (Figure A) or make an axe cut to split the cranium (Figure B).



- Pry the cut piece of cranium free from the underlying brain
- Extract the brain and spinal cord proximal to brain stem

## Sample quality

The quality of the sample will impact the probability of accurate results. However, there are times when a decomposing or infested sample may be all that is available.

### Decomposing samples

- Test sensitivity will be decreased for decomposing samples. They may still be submitted if there has been a suspected human or domestic animal exposure to rabies of a minimum Category II.
- Laboratory analysts will determine the extent of decomposition and declare the sample unfit for testing, if necessary.

### Infested samples

- It is important to remove any visible maggots prior to packaging the sample. Maggots will consume brain tissue during shipment, and there may be no sample left for testing by the time the package arrives at the laboratory.
- For the comfort and safety of laboratory staff, head or carcasses with evident flea or tick infestations should be sprayed with insecticide.

### Temperature control

- Temperature control is important to maintaining sample quality, but it is **not** necessary to freeze samples prior to shipping.
- The ice packs included with the sample should be adequate to keep the sample cool.
- If the sample has been frozen, ship it without ice packs. Frozen samples must be thawed before they can be processed, which will delay the time to obtain test results by a day or more.

## Sample submission – packaging and shipping

Submission of the sample for testing is a two-part process that includes packaging and shipping.

### Packaging

Each sample must be assigned a unique identifier, and a [Rabies Sample Submission Form](#) must be completed. Visit the [CVMA website for a video on how to package samples](#) or visit the [Rabies Guidance page of the CVMA website](#) and consult the guidance document, “Packaging of Samples.”

- The unique identifier is assigned by the submitter. If an alpha-numeric code is used, uppercase letters must be used. Durable, adhesive labels, printed with

indelible ink or commercially available identification tags can be used to attach the unique identifier to the primary package.

- Each Sample submitted to the laboratory must also be accompanied by its own Rabies Sample Submission Form, a fillable pdf that must be populated with required data. The fields where entry is mandatory are clearly marked on the form. Once completed, the data are submitted electronically to the CFIA, and a hard copy of the submission form is printed for inclusion with the shipment.
  - For more information, visit the [Rabies Guidance page of the CVMA website](#) and consult the guidance document, “Completing the electronic submission form.”
- When the data are received by the CFIA, an email will be transmitted confirming successful data transfer. An email will also be sent to the submitter once the sample has arrived at the lab, indicating that it has been received and is under test. When testing is completed, a report of analysis will be sent to all the email addresses identified by the submitter on the submission form.

The sample must be classified as a Biological Substance, Category B, and the mode of transportation must be selected to determine the appropriate packaging materials.

- For tissue known or suspected to be infected with rabies virus, the classification is “Biological Substance, Category B”.
- When shipping Infectious substances, the shipper must be [Transportation of Dangerous Goods \(TDG\)](#) certified for shipping Infectious Substances 6.2. Employers are responsible to issue a training certificate once their employee has received adequate training.

The package is then assembled and prepared for shipping by application of all necessary markings and labels. There are some specific needs with respect to packaging, some of which are dictated by Transport Canada, and others are requirements of the CFIA rabies laboratory. It is the responsibility of the shipper to ensure that all requirements of the Transportation of Dangerous Goods legislation are met. Packaging materials that would meet requirements for shipping Biological Substances, Category B, include:

- Primary package of 2 leak-proof plastic bags OR a screw cap plastic container and one plastic bag
  - If shipping brain tissue, or very small animals like bats, a screw-cap container, such as a urine specimen cup, is preferred to protect the tissue from being crushed.
  - Plastic bags are best for a packaging a head, or a small wildlife carcass.

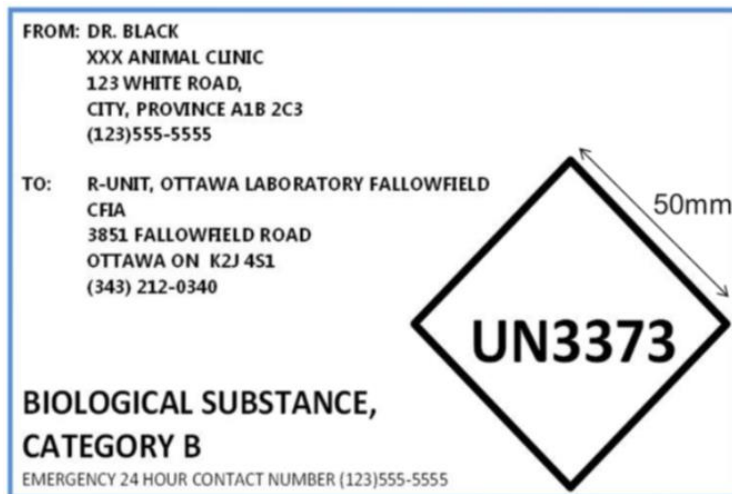
- If using an open-ended bag, secure it with a knot, or if using a zipper bag, seal the zipper, then fold over and secure the bag with moisture-resistant adhesive tape, such as duct tape.
- Place the first bag, or the screw cap container into a second plastic bag and seal. Affix the Unique Identifier to the outside of the second bag. This completes the primary package.
- Secondary package of a single leak proof plastic bag
  - If shipping by air, the secondary package must be a pressure capable receptacle to 95 kPa.
  - Do not use reusable pressure vessels as they will not be returned to the submitter.
- Absorbent material must be included to absorb any possible fluid leakage and to cushion the sample during transit (do not use Styrofoam, or similar granular packing material).
- Rigid ice packs
  - add several frozen rigid ice packs if the ambient shipping temperature is above 5 degrees Celsius.
  - If the outdoor temperature is above 30 degrees Celsius, use additional ice packs.
  - Do not use soft-walled gel packs as they may burst in transit.
- A sturdy outer container, such as a fibreboard box.
  - If shipping by air, this box will need to meet a 1.2 m drop-test requirement
- The sample must be identified with a unique sample identifier which must match the sample identification entered on the submission form
- The rabies Sample Submission Form serves as the package inventory.
- Outer container markings are also required to meet TDG regulations.

## Shipping

Shipping documentation must be completed and depends on the mode of transportation selected.

## Markings and labels

Below is an example of a label that contains all the required elements for shipping a “Biological Substance, Category B” sample.



It must include the name, address and telephone number of the shipper and the laboratory, in equal-sized font. CFIA recommends using block letters of at least 16 pt font for ease of reading. To expedite delivery of the sample to the rabies unit once the package arrives at the laboratory, be sure to include “R-UNIT” in the laboratory address.

The Proper Shipping Name, “Biological Substance, Category B” should be written at least 6 mm high with a line width of at least 2 mm.

The hazard mark, UN3373 diamond-on-point must also appear on the package. The minimum size of the diamond is 50 mm on each side. These labels can be purchased, or can be printed in-house, as long as these minimum requirements are met. Any additional labels applied by the shipping company must not occlude the required Transport Canada markings and labels or the name, address and phone number of the shipper or the laboratory.

For shipments by air, the outer shipping container must also be marked with the word “OVERPACK”.

The Proper Shipping Name and the UN number must also be stated on the Shipper’s waybill. If the shipment is by air, the packing instruction PI 650 must also be marked on the waybill.

## Shipping options

There are several choices available for shipping of samples, including:

- Couriers
  - Accept infectious substances for transport
  - Service to Ottawa Monday to Friday
- Airlines
  - Some airlines will accept infectious substances, check with carrier for policies
  - For air freight, notify the laboratory in advance

- Medical laboratory transport
  - Check with carrier for availability

The selection of carrier, the level of service available, and the costs involved vary from region to region. It is recommended to inquire directly with the shipping companies to determine the best options and any additional requirements they may have.

## Laboratory contact information and hours of operation

R-Unit, Ottawa Laboratory Fallowfield  
Canadian Food Inspection Agency  
3851 Fallowfield Road  
Ottawa ON K2J 4S1

Phone: (343) 212-0340

Fax: (343) 212-0202

Email: [cfia.rabieseast-rageest.acia@inspection.gc.ca](mailto:cfia.rabieseast-rageest.acia@inspection.gc.ca)

Hours of operation: Monday to Friday, 8 am to 4 pm Eastern time

## Weekend and holiday testing

Under certain circumstances, fresh samples from animals that have caused a Category III human exposure will be considered for testing on weekends and holidays. However, it should be noted that service from shipping companies is limited to Saturdays outside of the normal work week. For more information, visit the [Rabies Guidance page of the CVMA website](#) and consult the guidance document, “Weekend and Holiday testing.”

Samples from animals that have caused a category III human exposure will be tested outside of normal hours if the following criteria are satisfied:

- The animal is reasonably suspected of being rabid, based on a thorough investigation including consideration of the animal species involved, its behaviour at the time of the exposure, presentation of clinical signs consistent with rabies, geographical area, and for domestic animals, potential for previous contact with a rabid animal.
- The person has suffered a WHO Category III exposure (i.e., single or multiple transdermal bites or scratches, licks on broken skin, contamination of mucous membrane with saliva).
- There is a medical contraindication to receiving post-exposure prophylaxis.
- The attending physician or Medical Officer of Health provides the laboratory with the name and telephone number of a contact who is readily available to receive and act upon the result on the day of testing. The laboratory must be able to speak with an individual; test results will not be left on an answering machine.

Samples from suspect human cases will always be tested outside of normal working hours, if advance notice is given. Please call the Ottawa Laboratory directly at (343) 212-0340 if you are considering submitting human samples for testing.

## DOs and DON'Ts

Many of the DOs presented here have been already covered in the previous sections. However, there are some very important DON'Ts that need to be brought to your attention.

Remember that safety (of the public, the submitter, the transporter and the staff at the lab) is of utmost importance, as well as protecting the integrity of the sample.

	DO...	DON'T...
<b>Sample collection</b>	<ul style="list-style-type: none"> <li>• Be vaccinated against rabies</li> <li>• Use appropriate personal protective equipment (gloves, coveralls, face shield, etc.)</li> <li>• Humanely euthanize the animal without damaging the head or brain</li> <li>• Ensure all insects and parasites are dead or removed (e.g. treat for fleas and ticks, remove maggots, etc.)</li> <li>• Remove any sharp objects (e.g. needles, darts, bullets, etc.)</li> <li>• Submit portions of brain tissue collected bilaterally from hippocampus, cerebellum and brain stem if entire brain cannot be submitted</li> </ul>	<ul style="list-style-type: none"> <li>• Use any kind of power tool to remove the head</li> <li>• Alter the size of the head by removing the ears, snout, or by skinning</li> <li>• Use ether or freezing as euthanasia methods for small animals (e.g. bats)</li> <li>• Use buckets or cans as secondary packaging</li> </ul>
<b>Packaging</b>	<ul style="list-style-type: none"> <li>• Use absorbent materials for wrapping</li> <li>• Use rigid ice packs for cooling</li> <li>• Use a pressure-capable bag for secondary packaging if shipping by air</li> <li>• Give each sample its own primary package, unique identifier and submission form</li> <li>• Place brain tissue or small animals in rigid plastic containers</li> <li>• Attach a copy of the Rabies Sample Submission Form to the secondary package</li> </ul>	<ul style="list-style-type: none"> <li>• Use thin plastic bags that are easily punctured</li> <li>• Use opaque bags (e.g. orange biohazard bags)</li> <li>• Use wet ice, soft gel packs, frozen water bottles or dry ice</li> <li>• Wrap ice packs in newsprint</li> <li>• Freeze samples</li> <li>• Use Styrofoam or similar granular packing material</li> </ul>

	DO...	DON'T...
<b>Shipping</b>	<ul style="list-style-type: none"> <li>• Ensure all required markings and labels are on the outer shipping container</li> <li>• Ensure the correct address and contact number for the laboratory are included</li> <li>• Contact the carrier regarding shipping requirements and their schedule</li> <li>• Track your shipment and call the carrier if it does not arrive as expected</li> </ul>	<ul style="list-style-type: none"> <li>• Use biohazard labels on outer shipping containers</li> <li>• Use the word Rabies on the outer shipping container (use "R-Unit")</li> </ul>

## Final review

To review the process, the **submitter**:

- collects the sample and all necessary information to complete the submission form
- assigns a unique identifier
- completes and electronically submits a Rabies Sample Submission Form for each sample
- packages the sample in accordance with TDG requirements
- arranges for shipment to the CFIA laboratory

The **CFIA rabies laboratory in Ottawa**:

- receives the data from the Rabies Sample Submission Form; notification of successful data transfer will be provided by email.
- receives the sample; an electronic notification of receipt will be sent to the submitter that the sample is received and being tested
- issues a Report of Analysis; this will be emailed to the submitter and any other parties indicated on the submission form