



Our vision:

To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:

Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Key Learning Points

- General information on human cases in Canada
- Process
- Sample Types

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RABIES TESTING AT THE CFIA: HUMAN SPECIMENS



HUMAN RABIES CASES IN CANADA

Rabies in humans is rare in Canada. Since 1924, only 25 cases have been documented. The last reported case of domestically-acquired human rabies caused by a terrestrial mammal occurred in 1967 in Richmond, ON. Since that time, all cases but two were attributed to infection from bats. The remaining two cases (1984 and 2012) were acquired in the Caribbean, the result of dog bites.

Oral vaccination programs and other disease control strategies targeting certain terrestrial wildlife species have been effective in controlling (and in some cases, eliminating) rabies in a number of Canadian provinces. However, not all provinces have wildlife rabies control programs, and rabies control in bat populations is not possible. Even though bats carry their own distinct variants of the rabies virus, infected bats are able to transmit the virus to other mammals. These cases

are referred to as "spillover". Several spillover cases from bats into domestic animals are reported each year in Canada, with the potential to transmit the virus to humans. Thus, there is still the risk of exposure to rabies from animals throughout Canada, even in the absence of known epizootics in terrestrial mammals. In addition to a clinical picture consistent with rabies, examination of other factors is important for evaluating the possibility of rabies. Provide the laboratory with as much information as possible to assist in the selection of appropriate reagents for rabies diagnosis and virus typing.

Patient travel history: Travel to countries where rabies is endemic, particularly in dogs, should be considered a risk factor for rabies, even in the absence of a known exposure to a rabies vector. As the disease can also be caused by related *Lyssaviruses*, such as

Australian bat lyssavirus (ABLV), travel to countries free of rabies virus may still be a risk factor. The CFIA laboratory can provide advice on the distribution of rabies and other lyssaviruses within Canada and worldwide.

Potential rabies exposures: The incubation period for human rabies is normally between one and three months, but may vary between 10 days to more than a year. Recent or past contact with bats or other mammals is a risk factor for rabies, particularly in rabies endemic regions. Category III exposure is defined as single or multiple transdermal bites or scratches, licks on broken skin, or contamination of mucous membranes with saliva. Category II exposure is defined as nibbling of uncovered skin or minor scratches or abrasions without bleeding. Both types of exposure can result in transmission of the virus from an infected animal to a person.

CANADIAN FEDERAL LEGISLATION AND CFIA

Rabies is a federally reportable disease in Canada according to the *Health of Animals Act*, the *Health of Animals Regulations*, and the *Reportable Diseases Regulations*.

The CFIA is a federal agency, whose President reports to the Minister of Health. The CFIA provides diagnostic testing for suspect rabid animals and humans, and gathers statistics in order to provide national occurrence reports as well as to

meet international reporting responsibilities.

Though there are two CFIA laboratories which conduct rabies testing on suspect animals, only the Ottawa Laboratory Fallowfield (OLF) tests human suspect cases. The OLF is a WHO Collaborating Center and an OIE Reference Laboratory for Rabies and is accredited to the ISO 17025 quality standard. At the OLF, diagnosis of rabies

in humans may be performed by the following tests: fluorescent antibody test (FAT) on nuchal skin biopsy (antemortem) or brain tissue (postmortem) and reverse transcriptase polymerase chain reaction (RT-PCR) on skin, saliva or brain tissue. Virus variant typing by genetic sequence analysis or using discriminatory monoclonal antibody panels is also performed. The CFIA does not carry out serologic tests for rabies virus antibody.

PROCESS FOR SUBMISSION OF SAMPLES FROM HUMAN SUSPECT RABIES CASES

- Hospital notifies OLF of impending submission.
- After consultation with hospital regarding types of samples and urgency of testing, OLF arranges to have staff available for specialized testing, including overtime, if required
- OLF provides required documentation to hospital, including Rabies Specimen Submission Form (fillable pdf) and fax notification (page 4 of this bulletin), as well as any special instructions.
- Hospital collects the required samples; completes the Rabies Submission Form and submits it electronically; completes and faxes the notification page (page 4); packages samples and arranges for shipment to OLF.
- When sample is received at OLF, and email notification will be sent to the hospital.
- OLF conducts diagnostic testing; number and type of tests depends on the samples received.
- OLF reports results by phone to contact(s) listed on Rabies Sample Submission form as tests are completed.
- Official "Report of Analysis" will be emailed (and faxed, if requested) to hospital when all testing is complete.
- If rabies virus is detected, variant typing is conducted and results



Ottawa Laboratory Fallowfield

are communicated to the hospital.

- Hospital notifies appropriate Public Health authorities of results
- Hospital notifies OLF if further testing is required (e.g. testing of additional samples; monitoring of viral load in saliva)

NUCHAL SKIN BIOPSY

THE PROBABILITY OF DETECTING VIRUS IN PERIPHERAL TISSUE INCREASES WITH DISEASE PROGRESSION.

Skin at the nape of the neck containing many hair follicles is highly enervated. As such, rabies virus antigen may be detectable antemortem by FAT if centrifugal spread from the CNS has occurred. A full thickness biopsy of at least 5 mm and containing several hair follicles should be taken. Place moistened gauze in a container with the biopsy tissue, but do not wrap the tissue. Store at 4°C or -20°C until ready to ship.

At the lab, the skin is frozen and sectioned using a cryostat. Multiple sections are tested by the FAT. RT-PCR tests may also be performed. If rabies virus antigen is detected, the remaining skin will be processed to extract viral RNA and the rabies virus variant will be determined by sequence analysis. For these reasons it is important to submit a biopsy of adequate size to provide sufficient tissue for multiple tests.

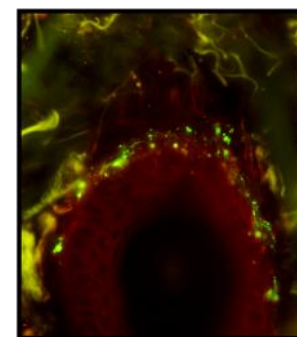


Image of rabies virus antigen in nuchal skin biopsy detected by FAT

SALIVA

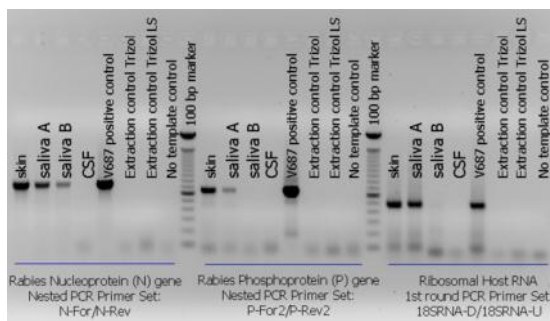


Image of amplified products from conventional RT-PCR analysis.

Saliva may be submitted for testing by conventional or real-time RT-PCR. Collect 2-3 ml of saliva directly into a sterile tube or aspirate and dispense into a sterile, leak-proof container. Do not add preservatives or additional material. Immediately freeze at -20°C, or lower, to prevent degradation of the viral RNA.

Serial samples taken at least 12 hours apart, and preferably 24 hours apart, will increase the sensitivity of the assay as

excretion of rabies virus may be intermittent in the early stages of the disease. The presence of bile or blood may interfere with the testing. However, if the only samples available are thus contaminated, they can still be submitted and the laboratory will attempt to test them.

DIAGNOSED RABIES CASES AND VIRAL LOAD MONITORING

If the patient is receiving medical care in an effort to sustain life, monitoring of the rabies virus load in the saliva may be requested.

A quantitative reverse transcriptase real-time PCR method (qRT-PCR) developed by Dr. Susan Nadin-Davis (J. Med. Virol. 2009, 81:1484) is the technique utilized. Saliva should be collected in a sterile tube and immediately frozen at -20°C , or lower, to prevent the degradation of the viral RNA. The sample volume should be at least 1 ml, and more, if available. Samples should be collected at

a frequency of every 24 hours. Clearly mark the date and time of collection on the individual tube.

Results will be expressed as cell culture infectious dose Equivalent Units (EU) per ml of saliva. EU is determined by interpolation of the test result on a standard curve of dilutions of known concentrations of rabies virus. Reports of these analyses will be issued by email to all the email addresses supplied on the "Rabies Sample Submission" form.

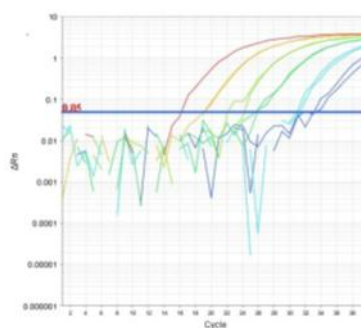


Image of amplification curves of serial 10-fold dilutions of rabies virus in qRT-PCR

SERUM AND CEREBRAL SPINAL FLUID (CSF)

The detection of antibodies specific for rabies virus in serum or CSF in a non-vaccinated person is diagnostic for rabies. However, serologic tests are not performed at the OLF. Samples of serum or CSF should be submitted for rabies virus antibody determination to the Public Health Agency of Canada National Microbiology Laboratory in Winnipeg, MB, or to the Central Laboratory of Public Health Ontario in Toronto, ON. Contact these laboratories directly for further information on sample collection and testing:

Public Health Ontario Laboratory:
<http://www.publichealthontario.ca/en/ServicesAndTools/LaboratoryServices/Pages/laboratory-location-and-contact.aspx>
(accessed January 2014)

PHAC National Microbiology Laboratory:
https://www.nml-lnm.gc.ca/guide2/pathogen_engview.php?refdiagID=24
(accessed January 2014)

Rabies virus is not haematogenous and will not be detected by RT-PCR in blood. CSF may contain low levels of rabies virus but when analyzed by RT-PCR the results are frequently inconclusive. Often, the CSF is not suitable to definitely declare a negative result as the dearth of cellular material makes it difficult to detect the control gene, β -actin, used in the procedure to indicate successful extraction and amplification reactions.

**CFIA WILL TEST
HUMAN SAMPLES
AT NO CHARGE.
TESTING IS
AVAILABLE DURING
ROUTINE BUSINESS
HOURS AND OUT-
OF-HOURS WITH
ADVANCE
NOTIFICATION.**

POSTMORTEM EXAMINATION OF CNS TISSUE

Fresh (not fixed) brain tissue should be submitted for testing. A small section (1 cm x 1 cm x 1 cm) from the brain stem is suitable for diagnosis. Additional tissues such as cerebellum, pons, medulla, hippocampus and spinal cord may also be submitted. Do not submit only cerebellum as it generally has lower levels of rabies virus antigen than other parts of the brain. Place the tissue into a leak-

proof container so it will not be damaged during transit. Store the sample at 4°C or lower until ready to ship. At the lab, the tissue will be examined by FAT. If rabies virus antigen is detected, the virus will be typed by staining with a panel of specific anti-rabies antibodies or by genetic sequencing.

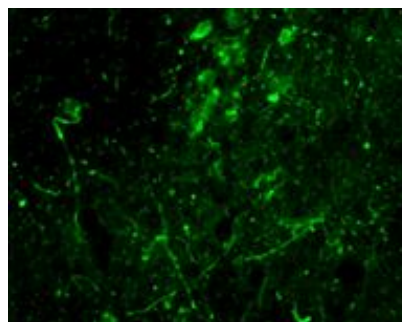


Image of rabies virus antigen in brain tissue detected by FAT



HUMAN SAMPLE SUBMISSION FOR RABIES TESTING

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For further information,
contact:

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

Mon-Fri 8 am-4 pm ET

Phone: 343-212-0340
Fax: 343-212-0202
E-mail: OLF_Rabies-LOF_Rage
@inspection.gc.ca

We're on the Web
www.inspection.gc.ca

If testing is required notify the laboratory at 343-212-0304.

Complete this page and FAX to 343-212-0202.

A "Rabies Sample Submission" form must also be completed. Submit electronically and enclose a paper copy with the shipment.

Please provide the following information so that we may cross-reference to the form:

Submitter Name:

It is the responsibility of the submitter to select a method of transportation that will ensure the delivery of the specimen. Courier service is available in Ottawa Monday to Friday and at special request on Saturdays. Purolator, Fedex or DHL service is NOT available on Sundays or holidays. To expedite processing, CFIA will arrange for transport from the courier depot to the laboratory on Saturdays. CFIA will arrange for transport from local airfreight facilities to the laboratory.

Courier Company or Airline:

Tracking Number:

Date and Time of Expected Arrival:

Patient Details:

Hospitalized: YES NO

Date of Illness Onset:

Animal Exposure History? NO or YES, describe including species and nature of exposure

Travel History outside of home province? NO or YES, list provinces and/or countries

Samples Submitted:

Date/time 1

Date/time 2

Date/time 3

Date/time 4

☐ Nuchal biopsy

☐ Saliva

☐ Postmortem brain/CNS tissue

Additional Comments: