Environment and Climate Change Canada
Environnement et Changement climatique Canada
Canada

Wildlife Surveillance Guidelines in Response to the Detection of SARS-CoV-2 in Farmed Mink in Canada

This document was developed with input from a working group consisting of Canadian wildlife, domestic animal, and public health experts, with representation from federal governments (Environment and Climate Change Canada, Canadian Food Inspection Agency, Public Health Agency of Canada), provincial governments, the Canadian Wildlife Health Cooperative, and academia.

Version 2, Date: February 17, 2022

Table of Contents

List of acronyms

AIHTS – Agreement on International Humane Trapping Standards

AVMA – American Veterinary Medical Association

BSC – Biosafety Cabinet

CBS – Canadian Biosafety Standard

CCAC – Canadian Council on Animal Care

CDC – Centers for Disease Control and Prevention

CFIA – Canadian Food Inspection Agency

CL2 – Containment Level 2

CL3 – Containment Level 3

CVO – Chief Veterinary Officer

CWHC – Canadian Wildlife Health Cooperative

cDNA – Complementary Deoxyribonucleic Acid

NCFAD – National Centre for Foreign Animal Disease

OIE – World Organisation for Animal Health

PCR – Polymerase Chain Reaction

PPE – Personal Protective Equipment

RT-PCR – Reverse Transcriptase Polymerase Chain Reaction

SARS-CoV-2 – Severe acute respiratory syndrome coronavirus-2

USDA - United States Department of Agriculture

VTM – Viral Transport Medium

1. Overall Objectives and Scope

The **purpose** of this document is to provide guidance to provincial wildlife, domestic animal, and public health authorities on recommended targeted surveillance approaches to be conducted in wildlife populations in the event of the detection of SARS-CoV-2 in farmed mink in Canada.

The **objectives** of targeted surveillance in response to a confirmed outbreak on a mink farm are to determine whether free-ranging wildlife on and around farms have been exposed to or infected with SARS-CoV-2, to identify potential SARS-CoV-2 transmission pathways at the human-domestic animal-wildlife interface, and to improve our ability to assess the risk of viral establishment in wildlife.

This evergreen document serves as a supplement to the Guidance for managing SARS-CoV-2 infections in farmed mink in Canada[1], and will be regularly updated as necessary to reflect new scientific information and surveillance data. This document does not cover the surveillance approaches for SARS-CoV-2 that may be applied to human populations or to captive or farmed wildlife species in Canada. While these guidelines were developed for the purposes of conducting targeted wildlife surveillance in response to an outbreak of SARS-CoV-2 on a mink farm, similar approaches may be used in response to laboratory confirmed cases of SARS-CoV-2 on other types of farms or premises depending on the scenario, species involved, and the estimated level of spillover risk. Based on available information and estimated level of risk, each province will decide on a case by case basis whether surveillance in those situations is warranted.

Furthermore, this document does not cover methods for opportunistic surveillance for SARS-CoV-2 in wildlife, given that overall objectives for opportunistic surveillance vary from that of targeted surveillance triggered by an outbreak. There are multiple research groups across Canada conducting opportunistic surveillance for SARS-CoV-2 and/or other coronaviruses in wildlife, and objectives, approaches, target species, and testing methods will vary amongst groups conducting this work. For more information on opportunistic surveillance and research studies in Canada, see **Annex IV**.

2. Background and Rationale

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is primarily spread in the human population through person-to-person transmission. While the virus has been detected in wildlife in Canada (see Animals and COVID-10 – Canada.ca / Les animaux et la COVID-19 – Canada.ca) and in the United States (see Animals and COVID-19 - CDC), the potential impact of SARS-CoV-2 on wildlife populations and the possible role of wild animal species in transmission and maintenance are all currently unknown.

The origin of SARS-CoV-2 has not been confirmed, but studies indicate the virus likely evolved from a mammalian host, possibly in Old World fruit bats[2]. North American bat species are known to host other coronaviruses[3], however betacoronaviruses, such as SARS-

CoV-2, have been found only in neotropical bat species in the Western Hemisphere, and not in temperate North American bat populations[3,4]. There are still many unknowns about how susceptible North American bats are to SARS-CoV-2 or novel variants, which may affect transmission, spread, and susceptibility. Thus, there is potential for SARS-CoV-2 to circulate within bat species in Canada should spill-over occur.

To date, numerous species of animals have been shown to be susceptible to SARS-CoV-2 infection under experimental conditions, and natural transmission of SARS-CoV-2 to animals has been documented for some pet, zoo, and farmed animals (e.g., mink), and to free-ranging mink and deer (see Animals and COVID-10 – Canada.ca / Les animaux et la COVID-19 – Canada.ca for species known to be susceptible to SARS-CoV-2).

Outbreaks of SARS-CoV-2 in both mink and personnel on mink farms have been reported in several countries including Canada, and have resulted in clinical disease, mortality, and/or large-scale culling of mink on affected farms (World Organisation for Animal Health (OIE) reports for SARS-CoV-2 events in animals)[5]. The virus can spread rapidly from mink-to-mink, and transmission of the virus back to humans, as well as to domestic cats and dogs on farms[6–8] has been documented. The rapid spread of SARS-CoV-2 among mink within a farm increases the opportunity for the virus to develop mutations, potentially resulting in the evolution of novel variants, as had occurred in Denmark in June 2020, when a mink-associated variant strain ("cluster 5") had spread from mink to farm workers, and subsequently into the local community, before being eradicated through intensive public health measures[9,10]. Furthermore, farmed mink are known to escape enclosures and barns[11], and studies in the USA have demonstrated that a large proportion of escaped farmed mink from outbreak farms were seropositive, and a low proportion of individuals also tested positive on polymerase chain reaction (PCR) test for SARS-CoV-2[12]. Hence, farmed mink may be a potential source of spread of virus from outbreak farms to wildlife[12]. To date, there has been one report of SARS-CoV-2 detected by real-time reverse transcriptase polymerase chain reaction (RT-PCR) testing in a free-ranging, wild mink in Utah, USA[13], as well as in two free-ranging (feral) American mink that had been trapped as part of ongoing programs for the elimination of mink in Spain[14]. In the positive wild mink from Utah, the sequence of the viral genome was indistinguishable from those obtained on a nearby affected commercial mink farm (United States report to the OIE on SARS-CoV-2 events in animals)[15].

There is concern that SARS-CoV-2 could spread from infected people and/or farmed animals into wild animal populations and establish a reservoir for the virus[16]. This concern is further highlighted with the finding by the United States Department of Agriculture (USDA) that a high prevalence of deer opportunistically sampled from archived serum samples were seropositive for SARS-CoV-2[17]. This study provides evidence that SARS-CoV-2 has spilled into wild deer populations in the USA, however it is unknown whether it continues to circulate in deer populations, and what the main modes of transmission are, thus further investigations are underway. Additionally, there is concern that some species could be susceptible to significant disease with possible population-level effects. Furthermore, susceptibility of wildlife species to SARS-CoV-2 may also vary over time with the evolution of novel variants of concern, potentially expanding host ranges, as has been demonstrated in laboratory mice which were not susceptible to the original strain of SARS-CoV-2, but found to be highly susceptible to the B1.351 and P.1 variants[18].

In the event of the detection of SARS-CoV-2 in farmed mink, targeted surveillance of wildlife would improve our ability to: evaluate whether free-ranging wildlife on and around outbreak farms have been exposed to or infected with SARS-CoV-2; identify potential SARS-CoV-2 transmission pathways at the human-domestic animal-wildlife interface; and improve our ability to assess the risk of viral establishment in wildlife. The detection of a positive case in wildlife would allow managers to evaluate the need for enhanced surveillance surrounding positive detections. For example, managers would need to evaluate the magnitude of spread, assess the potential for establishment of novel reservoirs, and identify factors associated with the spillover event in order to inform decisions surrounding mitigation, management, and prevention of subsequent spillover events at the farm-wildlife interface. Furthermore, the detection of SARS-CoV-2 in wild animals may necessitate subsequent investigations to evaluate routes of transmission and impacts on wildlife populations depending on the species involved. The establishment of novel reservoirs for SARS-CoV-2 in wildlife could potentially undermine public health efforts to eradicate the virus, not only by providing a potential source of re-emergence, but may also provide opportunities for the generation and spread of new variants[19,20].

Due diligence and public assurance are also needed to demonstrate that potential avenues for spread of SARS-CoV-2 in wild animal populations are being investigated, particularly given the heightened public perception of risk. This is of particular importance for helping provide assurance and building confidence in the safety and sustainability of cultural practices and traditional food systems, and meeting constitutional obligations to ensure Indigenous populations have access to safe wild foods.

3. One Health/Collaborative Approach

The term "One Health" recognizes connections between people, animals, plants, and the environment. In a One Health approach, multiple sectors communicate and collaborate to more effectively address shared health threats (World Health Organization (WHO) 2021)[21]. The two core objectives of the OIE Wildlife Health Framework[22] are to manage the risk of disease emergence at the human-animal-ecosystems interface, and to protect wildlife health. Given current knowledge on the epidemiology of SARS-CoV-2 at the human-animal interface, particularly in association with mink farms, it is essential for Canada to take a One Health approach to investigating and managing SARS-CoV-2 outbreaks in farmed mink (Guidance for managing SARS-CoV-2 infections in farmed mink in Canada)[1]. Plans for surveillance, investigation, and management must include the wildlife health sector from the onset. Should SARS-CoV-2 spill over and establish itself in Canadian wildlife populations, this could impact wildlife, domestic animal, and/or human health, thus multiple jurisdictions have responsibilities for managing this pathogen. The potential for SARS-CoV-2 to become established in a wildlife species, mutate, and spill back into human or domestic animal populations is unknown. Thus, timely detection and genomic analysis of positive wildlife cases in the proximity of infected mink farms would benefit not only wildlife authorities, but also public health and agricultural agencies tasked with demonstrating that all avenues for viral spread are being investigated. In Canada, multiple jurisdictions are responsible for management of response(s) to SARS-CoV-2, and thus rapid communications,

data sharing, and collaboration on surveillance and management activities will be mutually beneficial to wildlife, public health, agriculture, and conservation agencies. Exclusion of specific agencies could hamper their ability to exercise due diligence and meet regulatory responsibilities.

It is recommended that local/provincial wildlife, environment, public health, and agriculture authorities, as well as Indigenous communities and rights-holders within the vicinity of the mink farm outbreak, establish a collaborative team in order to share information, harmonize communications, and develop and implement targeted surveillance plans for SARS-CoV-2 at the human-animal-environment interface. This group should be established in advance so that all partners understand their roles and responsibilities for the investigation ahead of time, and appropriate communications and response plans can be implemented rapidly and efficiently, so that surveillance can be initiated as soon as possible. It is also important that each province/territory identify gaps in field surveillance or laboratory capacity, or deficiencies in resources, in order to identify whether and how partners in the federal government, Canadian Wildlife Health Cooperative (CWHC), or academia can provide additional support. Communication between potentially overlapping jurisdictions to define roles and responsibilities early in the planning phase is strongly recommended.

4. Approach to field methods and selection of target species

Contact information for CVOs and individuals leading targeted wildlife surveillance for each province/territory are listed in **Annex II** and **Annex III**, respectively.

4.1. Regulatory Considerations

All required provincial, federal, animal care, land owner/property, and rights-holder permits and permissions, where relevant, should be in place in advance of any trapping and handling of wildlife, and Canadian Council on Animal Care (CCAC) guidelines should be followed. Recognizing the need for wildlife agencies to form part of surveillance teams, permission for wildlife testing should be obtained from land owners and public health/agricultural agencies in charge of managing quarantines on affected premises. Furthermore, any wildlife sampling initiated or conducted by public health or agriculture officials on private property may also require appropriate approvals/permits by provincial/federal wildlife agencies depending on the species.

4.2. Field Reconnaissance

The landscape and species composition on and around affected premises should be evaluated by qualified field personnel to assess the suitability for trapping target species of wildlife. Depending on habitat suitability and accessibility, species composition, and estimated

numbers of targeted wildlife species surrounding the premise, we recommend that sampling of wildlife take place within 1-3 km around the affected property or location triggering targeted surveillance. Field reconnaissance is key for identifying and prioritizing interfaces/locations within the surveillance zone that have a higher risk of interaction between wild and farmed animals, or between wild animals and the affected premises (risk-based surveillance). Intensity of trapping effort should be higher in areas deemed to be higher risk for a spillover event, as well as for subsequent propagation should a spillover event occur. Wildlife surveillance cameras may also be deployed at key sites to evaluate wildlife species moving on and off farms, or entering areas seemed higher risk. Initial surveys of the area will also help identify species at risk or of conservation concern, for which additional measures involving surveillance or management may be warranted (and are beyond the scope of these guidelines).

4.3. Target species

Target species will vary with each scenario, location, province/territory, and objectives of the surveillance program, hence initial field reconnaissance is necessary to evaluate the species composition and estimated numbers/densities of potential target species within the sampling zone. In general, target species should include species (or their close relatives) that have been shown to be susceptible to SARS-CoV-2 via natural or experimental infection, as is illustrated in Table 1. The list of known susceptible species is continuously evolving as new information becomes available, hence refer to Animals and COVID-10 – Canada.ca / Les animaux et la COVID-19 – Canada.ca which is updated frequently. In addition to evidence of susceptibility, selection of target species should also be based on an assessment of the surveillance zone, habitat, species composition and densities; the degree of interaction of potential target species with the affected farm and humans; as well as estimated level of risk of impacts on target species, and their potential to subsequently propagate the virus, and/or become a reservoir[20].

Although there is currently no evidence that North American bats are susceptible to SARS-CoV-2 (Table 1, Section 2), provinces/territories may decide to sample bats opportunistically, during existing bat monitoring programs (e.g., ongoing rabies or white nose syndrome monitoring programs, or in collaboration with pest-control companies or rehabilitation facilities), particularly from populations near areas with high prevalence of COVID-19 in the human population. Should live bats be included as species to sample opportunistically in association with ongoing programs, particular care must be taken to prevent introduction of SARS-CoV-2 into bat populations[23,24], in addition to general wildlife handling guidelines[25].

Although feral/free-ranging domestic cats are not considered to be wildlife, they are a species of interest because of their tendency to interact closely with people and farms, their susceptibility to infection, and their potential ability to transmit SARS-CoV-2[26,27]. Thus, targeted or opportunistic sampling of feral cats is recommended if trapped within the surveillance zone, and provinces may develop plans in conjunction with relevant jurisdictions and stakeholders to sample and subsequently release, euthanize, or transport them to local shelters, as per local regulations and humane standards of practice.

Table 1. Examples of potentially susceptible wildlife and captive species in Canada, based on evidence of susceptibility (as of July 4, 2021) following natural or experimental exposure to SARS-CoV-2 in related species. Green and yellow icons indicate species that are potentially good candidates for targeted sampling based on their susceptibility, with black icons indicating species that have been shown not to be susceptible, or for which not enough information is known.

	Evidence of susceptibility based on natural or experimental infection	Examples of wildlife species in Canada	Potential candidate target species? ● Yes (mod to high) ● Yes (low to mod) ● No or unknown
Order Carnivora			
Mustelidae	**HIGH:** Mink[5,7,10,15,28-33], ferrets[5,28,34-38], Asian small-clawed otters[5]	All mustelids, including mink (wild/escaped/feral), otter, marten, weasels, ermine	
Felidae	**HIGH:** Domestic cats[5,28,34,39-45], pumas/cougars[5,28], lions[5,28,46,47], tigers[5,28,46,47], snow leopards[5,28]	Lynx, bobcats, cougars (also feral/domestic cats)	
Canidae	**LOW TO HIGH:** Raccoon dogs[28,48], domestic dogs[5,28,34,39,42]	Foxes, coyotes	
Mephitidae	**MODERATE:** Striped skunks[49,50]	Skunks	
Procyonidae	**LOW:** Raccoons[49,50]	Raccoons	
Order Rodentia			
Cricetidae	**MODERATE** to **HIGH:** Deer mice[49,51,52], Chinese hamster[53], golden Syrian hamsters[54,55,57], bank voles[57], bushy-tailed woodrats[49]	*Peromyscus* spp (e.g., deer mice, white-footed mice), voles, rats, muskrats	
Muridae	**LOW** to **HIGH:** Lab mice[18,56,58] and house mice[49] not/mildly susceptible to original SARS-CoV-2 variant, but lab mice highly susceptible to VOCs	House mice, old world rats	
Sciuridae	**NOT SUSCEPTIBLE:** Fox squirrels[49], Wyoming ground squirrels[49], black-tailed prairie dog[49]	Squirrels, chipmunks, prairie dogs, marmots	
Order Lagomorpha			
Leporidae	**HIGH:** New Zealand white rabbits[28,59] **NOT SUSCEPTIBLE:** Cottontail rabbits[46]	Rabbits, hares	
Order Artiodactyla			
Cervidae	**HIGH:** White tailed deer[28,60]	White tailed deer, mule deer, caribou, elk, moose	
Order Chiroptera			
Vespertilionidae (N. American bats)	**NOT SUSCEPTIBLE:** Big brown bats[61]	Numerous species across Canada	
Pteropodidae (Old World fruit bats)	**HIGH:** Egyptian fruit bat[28,37]	NONE in Canada	

The list of species in **Table 1** is not exhaustive. Other species, such as domestic pigs[34,37,62–64], cattle[28,65,66], and multiple avian species (ducks, geese, quail, chickens, turkeys)[28,34,37,67–69] have also been tested experimentally, but have been shown not to be susceptible to SARS-CoV-2. Multiple primate species have been shown to be susceptible[5,28,70–79], however they were not included in **Table 1** given that there are no wild free-ranging primates in Canada. For updated information on species susceptibility, please visit Animals and COVID-19 - Canada.ca / Les animaux et la COVID-19 - Canada.ca[80].

4.4. Trapping Methods

4.4.1. Lethal vs. live capture and release

Local or regional authorities should determine what trapping approach will be most appropriate for surveillance of target species in their jurisdiction. For target species that are abundant (e.g., not threatened or endangered), whole carcasses can be collected, either through live trapping and subsequent euthanasia, or through kill trapping. Lethal collection will prevent potentially positive animals from being released back into the wild, and allow for further confirmation of any positive detections via additional samples from the same specimen (e.g., complete necropsy, see **Section 6.4.2** below). Dependent on resources available, live capturing and subsequent euthanasia following CCAC or American Veterinary Medical Association (AVMA) guidelines is the preferred option over kill trapping to limit specimen degradation prior to sampling[81,82]. Considerations for live sampling and subsequent release should be evaluated on a regional basis, particularly for species of concern, non-target species, and in consideration of public sentiment. Furthermore, live sampling and release may be preferred during critical time periods. For instance, euthanasia of lactating females may cause inadvertent orphaning of juvenile offspring, which should be avoided. All animals released after sampling should be marked (e.g., with an ear tag or tracking device) prior to release, and appropriate guidelines must be followed to prevent transmission of SARS-CoV-2 into wild animals being handled and released[25]. All escaped farmed mink should be euthanized due to their increased potential for transmitting SARS-CoV-2 or other infectious diseases from farmed mink to surrounding wildlife. Farmed mink can generally be distinguished in the field from wild mink through pelt colour and characteristics, or morphological measurements (e.g., skull size)[83].

A combination of trapping methods can be used. Choice of traps/methods will be dependent on the targeted species, consideration for avoiding non-target species, and preferred approaches, expertise, and resources available within each jurisdiction. Chosen anesthesia or euthanasia protocols must follow accepted standards (follow guidelines from provincial / territorial animal care committee, CCAC or AVMA), and be conducted by qualified personnel only. Please note that there is a shortage of ethanol that is expected to extend into 2022, which may affect which methods are used. Trappers/field personnel need to adhere to jurisdictional regulations. Lethal trapping should be performed by licensed trappers using best practices as recommended by the Fur Institute of Canada[84]. All trapping must be conducted using traps that meet Canadian trapping standards and certification (by the Agreement on International Humane Trapping Standards (AIHTS)) for animal welfare and/or CCAC guidelines (for non-furbearers like small mammals)[85,86].

4.4.2. Level of effort

Regional knowledge of the target species and their home range is important for deciding level of effort and trap placement. If sufficient population and demographic data are available for the target species, epidemiological consultation is recommended to estimate sample size and distribution needed to obtain samples from a representative number of individuals in the population. Given that this knowledge will not be available for most target

species, it is recommended to sample animals from all targeted species within a 1-3 km radius of the affected farm, and trap for 7-10 nights (see below), during which a decline in trapping success usually occurs. In particular, recently escaped and trap-naïve mink could be captured around their farms of origin through effective trapping.

If trapping is permitted on the affected premises, traps should be placed around barns or outdoor barrier fences, and near carcass piles, debris piles, and areas with spilled feed or other attractants.

Traps need to be checked frequently, as outlined in approved animal care plans, or as recommended by CCAC guidelines[87], and at least one individual needs to be on call to respond to any reported trap issues and captures immediately.

4.4.3. General guidelines for fur-bearing mammals to maximize capture success relative to trapping effort

- Determine or estimate the home range size, available habitat, or attractants for target species within a 1-3 km radius area from the infected farm.

- Where possible, base home range size on the females of the target species, which tend to have smaller home ranges. This will ensure sufficiently high trap density to target both males and females.

- Limit the capture of non-target species through appropriate trap selection and placement, which will also leave more traps open for the capture of target species.

- The use of species specific baits, lures and visual attractants, as appropriate, can also aid in the capture of target species.

- Deploy 2-4 traps within the home range of each target species' in the surveillance area[88,89].
    - Be cautious about placing different traps for multiple species at the same trap site, which could dissuade wary animals from entering a trapping area.
    - Focal points for the placement, distribution and number of traps should be based on the location of suitable habitat and other attractants. Place traps in areas most likely to successfully capture the target species, e.g., in the case of mink: water/riparian areas, sewage ponds, domestic waterfowl/chicken farms.

- Run traps for 7-10 nights.
    - Number of days of trap deployment should ultimately be dependent on the level of capture success or continued risk presented by the affected farm.

- Trail cameras, placed in areas likely to be frequented by target animals, and in high risk areas (see Section 4.2), can help verify the effectiveness of trapping efforts and inform modifications needed to trapping strategies.

- **Trap deployment example:**
  - Target species: mink (escaped and wild)
  - Female mink density averages 1.4 animals/km^2 in the target region. Therefore, a 3 km radius from the infected farm (28 km^2 area) can support 20 home ranges.
  - 40-80 traps (20 home ranges X 2 to 4 traps) should be deployed across the target area for 7-10 days, or until trap success subsides.

4.4.4. Animals found dead

Wild animals found dead at any point during the field operations, regardless of the species or cause of death, should be prioritized for testing. In addition, animals found dead with no apparent cause, such as roadkill mink and other species, can offer quality samples when found in good condition and near a mink farm. Ideally, carcasses should be collected and brought to the designated diagnostic lab, or stored cold or frozen until shipped to the designated diagnostic lab (e.g., provincial diagnostic lab, CWHC for SARS-CoV-2 testing along with complete necropsy evaluation, if possible. Prior communication with the diagnostic lab is essential, to confirm whether swab testing may be required prior to carcass submission. Field personnel collecting wildlife carcasses should avoid direct contact by handling with a tool such as a shovel or tongs, and use caution to avoid skin puncture from claws or teeth. Appropriate personal protective equipment (PPE), including gloves and mask, should be worn (see *Wildlife and COVID-19: General Handling Guidelines*)[25]. Carcasses should be placed into leak-proof bags that are thick enough to minimize risk of puncture, and the bags sealed securely. The bag should be wrapped in absorbent material such as paper towels, and placed into a second bag similarly sealed, to prevent leakage and cross-contamination. Please refer to the Canadian Wildlife Health Cooperative guidelines[90] for detailed instructions on safely collecting and submitting carcasses of wild animals found dead to the appropriate lab[90].

4.4.5. Risk Mitigation Measures

Please refer to *Wildlife and COVID-19: General Handling Guidelines*[25] for guidance on protecting field personnel and minimizing the risk of transmission of SARS-CoV-2 to wildlife, between animals or sites, and preventing viral contamination of samples. Note that any animals sampled for SARS-CoV-2 should be released where they were trapped. Furthermore, all field personnel must comply with local/provincial public health restrictions, and adhere to quarantine protocols implemented by public and animal health officials when working on quarantined premises.

5. Logistics of sample collection and processing

Carcasses of trapped/euthanized animals in the field must be placed individually in sealed plastic bags labeled with their unique identification number, kept cold (preferably in refrigerator or frozen if there is no access to a fridge), and brought directly to the participating diagnostic, provincial/territorial, or partner lab within 24 hours of collection for

sampling and processing. For some provinces, carcasses will be pre-processed at the local provincial wildlife or partner lab, who can collect the required samples, following the protocols below (**Section 6**), and submit/ship the samples to the participating lab(s) for PCR and serological testing (**Section 8; Annex IV**). Ideally, if space and resources allow, once carcasses have been sampled, carcasses should be stored frozen until initial test results are obtained. If swabs are suspect positive (or "non-negative") for SARS-CoV-2 by PCR, then duplicate swabs or tissues must be shipped to the National Centre for Foreign Animal Disease (NCFAD) for confirmatory testing (**Section 8.3; Annex IV**). Carcasses of non-negative or positive animals should then be submitted to the designated pathology diagnostic lab/CWHC for full necropsy, in order to determine the extent of lesions, clinical significance of infection, and to acquire additional samples if needed. However, if resources or storage space are limited and carcasses cannot be stored until test results arrive, additional tissue samples of lung, nasal turbinates, small intestine, and large intestine should be collected and stored frozen for subsequent analysis in the event of a suspect positive or non-negative result. Appropriate biosafety protocols, including PPE, should be adhered to when handling and storing carcasses and samples (**Section 8.1**). Carcasses should subsequently be disposed of according to provincial guidelines and approved biosafety/biosecurity protocols.

Similarly, samples collected from live animals in the field must also be labeled with their unique identification numbers, kept cold (on ice and then transferred to refrigerator), and brought to the participating diagnostic, provincial/territorial, or partner lab within 24 hours of collection. Blood samples should be spun down within 24 hours at a maximum, with serum harvested and placed in labelled cryovials (and frozen until tested). Swabs and sera should be submitted or shipped to the designated lab(s) for PCR and serological testing. If swabs are suspect positive (non-negative) for SARS-CoV-2 PCR, then duplicate swabs must be shipped to the NCFAD for confirmatory testing (**Section 8.3**).

In some situations (e.g., remote field locations), batch shipments of carcasses and/or samples will be required. In this scenario, carcasses and/or samples should be frozen as soon as possible, and remain frozen until processed by the designated diagnostic lab(s).

6. Sampling protocols

6.1. Overview of sampling approach

Samples will be collected to test for evidence for the presence of, and previous exposure to, SARS-CoV-2. A summary of the sampling procedures is available in **Annex I**. It is imperative that the diagnostic laboratory(ies) (**Annex IV**) be consulted prior to initiating sampling, to ensure methods for sample collection, storage, and shipment are appropriate.

Similar approaches to sample collections from live and dead animals will be used, with a few differences (**Table 2**). The presence of SARS-CoV-2 will be evaluated using molecular screening methods (RT-PCR). Recommended samples for PCR testing include nasal (carcasses only), oropharyngeal, and rectal swabs, collected in duplicate, and placed in individual cryovials containing appropriate viral transport media (**Section 6.3**). Nasal swabs

have been shown to have higher viral loads compared to oral or rectal swabs in clinically infected mink (Himsworth personal communication).

There is a short period in which viral shedding occurs, limiting the efficacy of PCR to detect infection. Therefore, previous exposure to SARS-CoV-2 will be tested using serological assays measuring antibodies to the virus. The most ideal sample for serology is serum whenever possible, however in dead animals, other samples that can be submitted include filter paper strips soaked in heart blood, or heart blood collected in a red top tube (**Section 6.4**).

For some species, sampling will require chemical immobilization. Appropriate methods and chemicals for such immobilization will depend on the species, and will require trained personnel. Please adhere to any biosafety considerations by institutions and provinces/territories. For more information on required training, and on safe chemical immobilization methods, refer to the Canadian Association of Zoo and Wildlife Veterinarians[91].

Table 2. Overview of samples to collect from carcasses or live animals. Diagnostic labs should always be consulted prior to sampling to ensure that preferred practises are followed.

Tissue/Sample Type	Sampled from carcasses	Sampled from live animals
Nasal swab* (or nasal wash in small mammals)	Yes - duplicate	No
Oropharyngeal swab*	Yes - duplicate	Yes - duplicate
Rectal Swab	Yes - duplicate	Yes - duplicate
Sample for serology: (e.g., serum, blood-soaked filter paper strip, or heart blood in red top tube)	Yes (serum or filter paper preferred)	Yes (serum preferred)
DNA sample (mink only - see text for options)	Yes	Yes
Freeze remainder of carcass until PCR results are received (for full necropsy if PCR-positive for SARS-CoV-2)**	Yes	Not applicable

**If preferred, the nasal sinus and oropharyngeal cavity can be swabbed with the same swab. If this is done, please indicate this on the sample vials and datasheets*

*** In cases where carcasses cannot be stored frozen until PCR test results are obtained, samples of lung, nasal turbinates, small intestine, and large intestine should also be collected and frozen.*

6.2. Record

- Use data sheet in **Annex VI** and enter the requested information (e.g., animal identification number, species, age, sex, body condition, mass, location (e.g., geographic coordinates/landmark/city), name(s) of individual(s) doing sampling, sampling date, observations (e.g., signs of nasal discharge, respiratory or other illness). Ideally, location data will be sufficiently detailed to determine proximity to farm or other significant sites in the sampling zone.

- Transfer data into excel spreadsheets and send electronically to diagnostic lab. Alternatively, scan and submit datasheets electronically. Data sheets may also be submitted along with sample shipment, but it is recommended that copies (or scans) be made as back-up.

6.3. Nasal, oropharyngeal and rectal swabs

- **General notes on swabbing:** Remember to change gloves, and disinfect the work area in between animals. In addition to ensuring the handler's safety, it is also important to avoid cross-contamination when collecting samples to make sure they are suitable for subsequent analysis. Open the plastic applicator swab envelope on the 'stick' end (i.e., do not touch the sterile polyester-end), and remove the swab. If the applicator end of the swab touches anything other than the intended sample, discard and use a new swab. After the sample is collected (see below), insert the swab into the vial containing viral transport medium (VTM) or other appropriate medium (e.g., lysis buffer for diagnostic labs that do not accept samples in VTM). Break the swab tip off into the tube, by prying it against the lip of the tube (being careful not to generate aerosols). Do not use scissors to cut the swabs off, to avoid spillage and contaminating vials with the content of other vials or swabs (cross-contamination). Close vial tightly, immediately after putting the swab in it, and consider wrapping with parafilm to secure lid

- **Nasal swabs**: Insert swab into one nostril, deep into the nasal turbinates (at the very back of the nasal passage), and rotate swab against the mucosal surface to obtain sample. Then break swab into cryovial containing media. For small mammals (e.g., rodents), a nasal wash can be collected in lieu of a nasal swab (pipet ~100 ul of media into nostril, suck up and dispense back into nostril several times and then place in cryovial containing media). Collect duplicate swabs in separate vials to allow for confirmatory testing of non-negative samples. (Note: nasal swabs or washes might not be feasible or possible to conduct safely in live animals unless the animal is anesthetized, hence these can be excluded when sampling live animals.)

- **Oropharyngeal swabs**: Open the mouth with gloved hands and swab the applicator along the mucosa at the back of the throat/pharyngeal area, behind the base of tongue. Place swab into cryovial containing media. *PLEASE note that many wild carnivores can also be rabies reservoirs, and use caution to avoid puncturing gloves. Collect duplicate swabs in separate vials to allow for confirmatory testing of non-negative samples.

- **Rectal swabs**: Insert the swab into the rectum with gloved hands and rotate/swab along the mucosal wall. Place swab into cryovial containing media. *PLEASE note that wild carnivores can carry zoonotic parasites, and change gloves and wash hands, if possible, or use hand sanitizer after handling fecal matter. Collect duplicate swabs in separate vials to allow for confirmatory testing of non-negative samples.

- NOTE: To help reduce cost of materials and lab testing, a single swab can be used to swab the nasal sinuses and oropharyngeal cavity (Himsworth, personal communication). If this is done, please indicate this on the sample vials and datasheets. A single swab is not appropriate for oral and rectal samples.

- Ensure all cryovials are clearly labelled with the animal ID and swab type, place the vials in a plastic re-sealable bag, and immediately place on ice or coolers with ice packs until samples can be transferred to proper cold storage (see below).

6.4. Samples for serology

6.4.1. From live animals:

- After collecting blood through appropriate venipuncture techniques for the species being sampled, blood should be placed in a red top tube and held on ice packs in a cooler until transported to the lab. Blood samples should be spun down as soon as possible, with serum harvested and placed in labelled cryovials, and frozen at -20°C until tested.

- Alternatively, filter paper strips can be submitted for serology. Whole blood collected by syringe can be dripped onto the long skinny end of 2 filter paper strips.

- Label the large end of the strip with the animal ID. Allow filter paper strips to dry on a sterile towel or on a portable drying rack with appropriate containment (for 24 hours). Do not place the strip in direct sunlight to dry. Then place in labeled whirl pack bags, and store frozen at -20°C.

6.4.2. From carcasses:

- All partial or full necropsies should occur at a CL2 facility.

- Label the vials (animal ID and "heart blood") in preparation for sampling to help minimize handling and contamination of the outside of the sampling containments or other tools.

- Use a fresh set of gloves, a new scalpel blade, clean forceps, and a cleaned/disinfected workspace and equipment between each animal to avoid cross-contamination.

- After cutting open the rib cage (using scissors or bone cutters), use a needle and syringe, or carefully cut open the heart, and collect heart blood into a red top tube, without

contaminating the heart blood with tissues or other fluids. Place sample in vial and then in the larger plastic re-sealable bag with other samples.

- If collecting heart blood using filter paper strips, excess blood can be dripped onto 2 filter paper strips, or the long skinny end of each strip can be dipped directly into the blood until the entire length is filled. Alternatively, uncontaminated chest cavity fluid can also be used.

- Label the large end of the strip with the animal ID. Allow filter paper strips to dry on a sterile towel or on a portable drying rack with appropriate containment (for 24 hours). Do not place the strip in direct sunlight to dry. Then place in labeled whirl pack bags, and store at -20°C and, if not available, any freezer.

- Complete necropsies are recommended on carcasses that are suspect or confirmed positive for SARS-CoV-2. All suspect positive carcasses should be submitted immediately for full necropsy, while other carcasses should be retained frozen until test results are obtained. Necropsies must be performed in a facility that meets the requirements for Containment Level 2 (CL2) as specified by the Canadian Biosafety Standard should include gross examination and sampling of a complete set of tissues for formalin fixation and subsequent histopathologic examination, as well as collection of select tissues to freeze at -80 C for possible further testing. A complete necropsy protocol and tissue checklist for suspect positive animals is provided in **Annex VI**.

- If carcasses cannot be retained frozen until PCR test results are obtained, additional tissue samples should be collected. Use forceps and scissors to excise an approximately 0.5 cm diameter x 1 cm long section from a lung lobe, and place it into a cryovial or whirlpak bag. Use a similar technique to retrieve sections of small and large intestine, and place in separate vials or whirlpak bags. To collect nasal turbinates in larger animals, reach deep into a nostril with a pair of small forceps and grab a piece of the bony tissue. Alternatively, and especially in smaller animals, the nasal cavity can be entered with a pair of rongeurs or a small saw, and use forceps with scissors or a scalpel to retrieve a piece of the scrolled turbinates. All samples should be frozen as soon as possible.

6.5. Genetics sample (MINK ONLY)

- For all live or dead mink, also collect a DNA sample, to enable analysis of degree of hybridization between wild and captive mink.

- **From carcasses**, sections of skin, liver, or muscle tissue (the size of a pencil eraser) can be placed in cryovials or whirlpaks and held on ice until stored in a freezer prior to shipping. Alternatively, samples can be placed in a cryovial of lysis buffer.

- **Samples from live mink** should also include a DNA sample. Appropriate tissue from live mink for DNA sampling includes > 30 hairs with roots stored dry at room temperature in a coin envelope, a blood sample stored frozen or in lysis buffer, or a cheek swab stored in lysis

buffer. Hair samples in coin envelopes should be placed in a re-sealable bag or sealed plastic containers with silica desiccant.

- See **Annex IV** for primary contact for submission of genetic samples from mink.

6.6. Carcass disposal

Ideally, carcasses should be retained frozen, until PCR results are available. It is recommended that any animals that are confirmed positive for SARS-CoV-2 undergo a necropsy to assess for possible lesions and to provide insight on the possible pathogenesis of viral infection in the species. Carcasses should be clearly identified as SARS-Co-V-2 positive, and necropsies should be performed under appropriate biosecurity conditions. Whether or not necropsies are performed, carcasses should be disposed of following institutional, provincial or territorial regulations for biohazardous waste.

7. Sample handling and transport

7.1. Sample preservation

- It is imperative that the participating diagnostic lab(s) be consulted about their preferred practices for appropriate sampling handling, storage, and shipping, prior to the commencement of sampling. See list of diagnostic labs for PCR testing and for serological testing in **Annex IV**.

- All blood, tissue, and swab samples should be kept cold (e.g., on ice, or ice packs in coolers) as soon as they are collected, and transferred to a refrigerator (2-4°C) if they can be shipped on ice packs within 24 hours.

- If samples are being batch-shipped, then freeze samples as soon as possible. Once frozen, the samples must remain frozen until analyzed, as coronaviruses are susceptible to freeze/thaw cycles.
    - Hence, these samples must be delivered frozen, at minimum on solid ice packs (not loose ice cubes), or on dry ice (if shipper has appropriate TDG training), and shipped to the designated diagnostic lab(s) for next day delivery.

- Note: If more convenient, samples that are in lysis buffer vials can be stored and shipped separately at room temperature, however they can also be combined with the frozen sample shipment.

7.2. Shipping instructions

- Prior to shipping samples, always consult with the receiving diagnostic lab regarding appropriate shipping protocols, and notify them whenever samples will be shipped.

- Register online to use CANUTEC's free, 24-hour emergency telephone number on your dangerous goods shipping documents.

- Samples are to be shipped according to packaging Type P650 requirements. A Type P650 packaging shall consist of inner packaging comprising of a leak-proof primary receptacle inside a rigid secondary leak-proof packaging. An outer packaging must have at least one surface with a minimum dimension of 100 mm x 100 mm.

- When transporting liquid substances, absorbent material must be placed between the primary receptacle and the secondary packaging in sufficient quantity to absorb the entire contents of the primary receptacle so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging. When transporting solid substances, if there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then a packaging suitable for liquids, including absorbent materials, shall be used.

- As per current recommendations by Transport Canada [92], the Minister of Transport has issued Temporary Certificate TU-0764.1 that authorizes the handling, offering for transport, or transporting of dangerous goods that are COVID-19 test samples under subsection 31(2.1) of the Transportation of Dangerous Goods (TDG) Act. This certificate takes into account existing safety and TDG requirements and the fact that some health care professionals may not be trained in accordance with the TDG Regulations.

- *In the absence of appropriate TDG training*, the following procedure under the guidance of Temporary Certificate TU-0764.1 is to be used:

    - When samples must be shipped frozen, solid ice packs are to be used as a refrigerant.

    - Samples can be shipped in a styrofoam cooler (primary packaging) inside a rigid cardboard box (secondary packaging), on solid ice packs. Sufficient absorbent material must be placed between the Styrofoam cooler and cardboard box.

    - Sample tubes and whirlpack bags should be placed within re-sealable bag(s), and placed inside the styrofoam cooler and surrounded with ice packs.

    - Tape the styrofoam cooler closed in 2-3 spots.

    - On the outside of the box, label with UN3373 for Biological Substance Category B (see label template in **Annex VII**). Include the shipping label "Biological Substance Category B", the words "Test Samples - COVID-19", the expression "TU 0764" or "Temporary Certificate - TU 0764", to/from labels, the words: "In Case Of Damage Or Leakage Immediately Notify Local Authorities and" followed by the 24-hour contact telephone number.

- Note: If deemed more convenient, samples that are in lysis buffer can be shipped separately at room temperature, also using a styrofoam cooler within a cardboard box.
  - Place samples in re-sealable plastic bags, and place bags into the cooler. Surround the cooler with adequate absorbent material. Label the outer cardboard box with UN3373. Include the shipping label "Biological Substance Category B", the words "Test Samples - COVID-19", the expression "TU 0764" or "Temporary Certificate - TU 0764", to/from labels, the words: "In Case Of Damage Or Leakage Immediately Notify Local Authorities and" followed by the 24-hour contact telephone number.

- *When the shipper/consignor possesses appropriate TDG training*, they may choose to use dry ice as a refrigerant for samples that must remain frozen. The following procedure for shipping samples on dry ice is to be used:

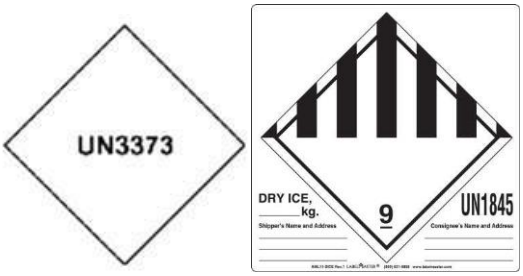
  - Ensure all relevant TDG regulations and IATA/ICAO technical instructions are followed.

  - Samples can be shipped in a styrofoam cooler (primary packaging) inside a rigid cardboard box (secondary packaging), on dry ice. Sufficient absorbent material must be placed between the Styrofoam cooler and cardboard box.

  - Sample tubes and whirlpack bags should be placed within sealed plastic bag(s), and placed inside the styrofoam cooler and surrounded with dry ice.

  - Do not seal the lid completely with tape to allow the dry ice to evaporate through the cracks. The packaging must permit the release of carbon dioxide gas and prevent a build-up of pressure that could rupture the packaging.

  - On the outside of the box, label with UN3373 for Biological Substance Category B and UN1845 for dry ice. Include the shipping label "Biological Substance Category B", the words "Test Samples - COVID-19", to/from labels, the words: "In Case Of Damage Or Leakage Immediately Notify Local Authorities and" followed by the 24-hour contact telephone number.



8. Diagnostic Testing

8.1. Biosafety advisory for diagnostic laboratories

Lab biosafety guidelines for labs conducting non-propagative diagnostic or research on SARS-CoV-2 follow the Biosafety Advisory: SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus 2 published by the Public Health Agency of Canada [93].

Non-propagative laboratory diagnostic activities performed on primary specimens (e.g., tissue, serum, blood, swabs, carcasses), must be carried out in a facility that meets the requirements specified in the Canadian Biosafety Standard (CBS) for a CL2. Personnel carrying out diagnostic and laboratory activities must follow, at minimum, good microbiological laboratory practices in work areas where primary specimens are handled. Relevant diagnostic activities for which routine practices and universal precautions are recommended include: sample preparation for nucleic acid extraction; receiving and storage of samples from collaborating partners; preparation of specimens for packaging and distribution to diagnostic laboratories for additional testing.

- Where aerosols may be produced during diagnostic activities with primary specimens under investigation for COVID-19, it is recommended that laboratories also implement additional biosafety recommendations. These include the use of a biosafety cabinet (BSC). When a BSC is available, the following biosafety recommendations are recommended:

- A lab coat, gloves, goggles / face shield and a respirator with proper fit testing should be worn when handling primary specimens.

- Certified BSCs are used for procedures that may produce infectious aerosols or droplets and activities involving open vessels of infectious material (i.e., not yet inactivated).

- Centrifugation of primary specimens is carried out in sealed safety cups, or rotors, that are loaded/unloaded in a BSC.

- All laboratory workspaces, surfaces, and materials must be wiped down with 70% ethanol to chemically disinfect and ensure sufficient inactivation of any SARS-CoV-2 containing material following any relevant laboratory work.

Please note that for any propagative *in vitro* or *in vivo* activities (e.g., virus isolation, experimental inoculations of animals, sample collections from experimentally infected animals), a minimum containment level 3 (CL3) is required.

Please refer to Biosafety Advisory: SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus 2[93] for further guidance on protecting personnel and minimizing risk of viral contamination or transmission.

8.2. Diagnostic laboratories

A list of diagnostic labs in Canada with capacity to conduct RT-PCR and/or serological testing for SARS-CoV-2 in wildlife samples is presented in **Annex IV**, respectively. It is likely that samples for RT-PCR and serology will be conducted in separate labs, depending on the province.

Diagnostic labs conducting RT-PCR for SARS-CoV-2 should be using validated protocols consistent with international standards. Several protocols using different primers or combinations of primers exist (e.g., primers targeting RdRp and E genes[94], or primers targeting nucleoprotein genes[95]. Labs across Canada may vary in their selection of primers for PCR testing. Definitions for non-negative and confirmed positive cases in wildlife are as described by Canadian Food Inspection Agency (CFIA, Interim Guidance for laboratories testing animals for SARS CoV-2)[96].

For serologic assays, which detect antibodies to SARS-CoV-2, commercial assays exist, and have been validated for use in human sera. These assays, as well as novel in-house assays being developed by various labs in Canada, are currently being validated for use in domestic and wild animals. Thus, determination of possible exposure to SARS-CoV-2 in wildlife species may require further assay validation for each species, and this is a subject of active research in Canada.

To increase the amount of information obtained as part of targeted surveillance for SARS-CoV-2, duplicate samples can also be used for testing for other coronaviruses, using a pan-coronavirus PCR assay[97]. A list of labs in Canada with the capacity to analyze samples for other coronaviruses can be found in **Annex IV**.

8.3. Confirmation and reporting of results

As per the CFIA's Interim Guidance for Laboratories Testing Animals for SARS-CoV-2[96], a non-negative PCR result from the diagnostic laboratory must be confirmed by the National Centre for Foreign Animal Diseases (NCFAD), CFIA, in Winnipeg. Duplicate samples and complementary DNA (cDNA) from the original sample should be provided. The address for shipping is 1015 Arlington St., Winnipeg, MB, Canada. R3E 3M4, made attention to Dr. Kathleen Hooper-McGrevy, Diagnostic Coordinator. Please include a shipper's declaration in box with itemized list of contents, shippers name and contact info. Once prepared to ship, please email the waybill number of the parcel to Dr. Kathleen Hooper-McGrevy at

kathleen.hoopermcgrevy@canada.ca and call ahead to the following number: 204-789-5048. Alternate contact: Dr. Carissa Embury-Hyatt carrisa.emburyhyatt@canada.ca.

A confirmed positive finding of SARS-CoV-2 in a wild animal meets the criteria for reporting to the OIE in accordance with the OIE Terrestrial Animal Health Code (Interim Guidance for Laboratories Testing Animals for SARS-CoV-2[96]). See **Annex V** for details on reporting a positive SARS-CoV-2 detection. Results should not be reported publicly until confirmation by the NCFAD and notification of the OIE have been completed.

A positive serological test provides evidence that the animal was previously exposed to SARS-CoV-2, and provides no information on current infection status. Hence, tests that are positive on serology in the absence of confirmed positive PCR tests do not require immediate notification to the OIE, but rather a letter or report indicating findings of interest.

8.4. Data sharing and dissemination of results

For wildlife, which do not typically fall under the jurisdiction of the provincial or territorial Chief Veterinary Officer (CVO) or public health department, it is strongly recommended that each province/territory establish open lines of communication to ensure results of wildlife testing at diagnostic labs, and wildlife confirmatory testing at NCFAD, are disseminated to the appropriate federal, provincial, and territorial agencies responsible for wildlife, domestic animal, and public health, as well as other key stakeholders. For more information on reporting see **Annex V**.

For any wildlife sampled in association with Indigenous communities, it is particularly important to share and communicate results in a culturally-appropriate manner, preferably using cross-cultural and collaborative approaches to generate information specific to the communities involved. In these cases, sample collection, data sharing, and communication should be discussed openly with Indigenous communities early, during the planning for targeted sampling.

8.5. Follow-up actions after confirmed SARS-CoV-2 detection in wild animals

It is recommended that a plan for responding to a confirmed positive case in a wild animal is developed by the province/territory prior to embarking on a targeted surveillance program for SARS-CoV-2 in wildlife. It is beyond the scope of this document to provide specific response guidelines, however, at minimum, immediate expanded wildlife surveillance around the point of detection is recommended.

9. Guidelines for Other Scenarios for Wildlife Surveillance for SARS-CoV-2

9.1. Other point-source outbreaks

Outbreaks of SARS-CoV-2 infection in other scenarios may also trigger a targeted wildlife surveillance program. These other scenarios could include wildlife rehabilitation centres; zoos, wildlife parks, or aquaria; non-mink fur farms; or other farms (e.g., cervid farms). In general, these guidelines for mink farms may also be applicable to other scenarios, with the added recommendation that surveillance for wildlife within facility premises be included.

9.2. Scanning/opportunistic wildlife surveillance

Opportunistic surveillance for SARS-CoV-2 in wildlife could be undertaken in collaboration with wildlife researchers, hunters/trappers, wildlife rehabilitation centres, and wildlife diagnostic laboratories. One scenario for consideration would be surveillance of peri-domestic and urban wildlife within areas of high prevalence of SARS-CoV-2 infection. It should be noted that any non-negative tests obtained by researchers must also be confirmed at the NCFAD, and confirmed positive results must be reported to the OIE, as described above (Interim Guidance for Laboratories Testing Animals for SARS-CoV-2)[96]. As an alternative or complementary approach to animal surveillance, environmental sampling (e.g., water samples in proximity to point source outbreaks) could be used to identify possible viral contamination from a specific site. A list of researchers currently conducting opportunistic surveillance or research related to SARS-CoV-2 in wildlife in Canada is provided in **Annex IV**.

10. References

1. *Guidance for managing SARS-CoV-2 infections in farmed mink in Canada*. (2021).
2. Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579,** 270–273 (2020).
3. Olival, K. J. *et al.* Possibility for reverse zoonotic transmission of SARS-CoV-2 to free-ranging wildlife: A case study of bats. *PLos Pathog.* **16,** e1008758 (2020).
4. Anthony, S. J. *et al.* Coronaviruses in bats from Mexico. *J. Gen. Virol.* **94,** (2013).
5. Health, W. O. for A. COVID 19: Events in Animals. (2020). Available at: https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/events-in-animals/. (Accessed: 29th June 2021)
6. Burkholz, S. *et al.* Paired SARS-CoV-2 spike protein mutations observed during ongoing SARS-CoV-2 viral transfer from humans to minks and back to humans. *bioRxiv* **preprint,** (2021).
7. Boklund, A. *et al.* SARS-CoV-2 in Danish Mink Farms: Course of the Epidemic and a Descriptive Analysis of the Outbreaks in 2020. *Animals* **11,** 164 (2021).
8. Hosie, M. J. *et al.* Anthropogenic infection of cats during the 2020 COVID-19 pandemic. *Viruses* **13,** 185 (2021).
9. Control, E. F. S. A. and E. C. for D. P. and *et al.* Monitoring of SARS-CoV-2 infection in mustelids. *EFSA J.* **19,** 6459, 68 pp. (2021).
10. Hammer, A. S. *et al.* SARS-CoV-2 Transmission between Mink (Neovison vison) and Humans, Denmark. *Emerg Infect Dis* **27,** 547–551 (2021).
11. Kidd, A. G., Bowman, J., Lesbarreres, D. & Schulte-Hostedde, A. I. Hybridization between escaped domestic and wild American mink (Neovison vison). *Mol. Ecol.* **18,** 1175–1186 (2009).
12. Shriner, S. A. *et al.* SARS-CoV-2 Exposure in Escaped Mink, Utah, USA. *Emerg. Infect. Dis.* **27,** 988–990 (2021).
13. DeLiberto, T. & Shriner, S. *Coronavirus disease 2019 update (536): animal, USA (UT) wild mink, 1st case*. (2020).
14. Aguilo-Gisbert, J. *et al.* First description of natural SARS-CoV-2 infection in two feral American mink (Neovison vison) caught in the wild. *Animals* **11,** 1422 (2021).
15. (ProMED), I. S. for I. D. Coronavirus disease 2019 update (536): animal, USA (Utah) wild mink, first case. (2020). Available at: https://promedmail.org/promed-post/?id=8015608. (Accessed: 29th June 2021)
16. GLEWS+. *SARS-CoV-2 in animals used for fur farming Risk assessment SARS-CoV-2 in animals*

used for fur farming. (2021).

17. Chandler, J. C. *et al.* SARS-CoV-2 exposure in wild white-tailed deer (Odocoileus virginianus). *bioRxiv* **preprint,** (2021).

18. Montagutelli, X. *et al.* The B1.351 and P.1 variants extend SARS-CoV-2 host range to mice. *bioRxiv* (2021). doi:https://doi.org/10.1101/2021.03.18.436013

19. Harrington, L. A. *et al.* Wild American mink (Neovison vison) may pose a COVID-19 threat. *Front. Ecol. Environ.* **19,** 266–267 (2021).

20. Delahay, R. J. *et al.* Assessing the risks of SARS-CoV-2 in wildlife. *One Heal. Outlook* **3,** 7 (2021).

21. Organisation, W. H. Tripartite Zoonoses Guide: Operational tools and approaches for zoonotic diseases. (2021). Available at: https://www.who.int/initiatives/tripartite-zoonosis-guide. (Accessed: 26th July 2021)

22. Health, W. O. for A. *OIE Wildlife Health Framework 'Protecting Wildlife Health to Achieve One Health'*. (2021).

23. Cook, J. D., Grant, E. H. C., Coleman, J. T. H., Sleeman, J. M. & Runge, M. C. Risks posed by SARS-CoV-2 to North American bats during winter fieldwork. *Conserv. Sci. Pract.* e410 (2021). doi:https://doi.org/10.1111/csp2.410

24. Runge, M. C. *et al. Assessing the risks posed by SARS-CoV-2 in and via North American Bats - Decision framing and rapid risk assessment*. (2020).

25. *Wildlife and SARS-CoV-2: Handling Guidelines*. (2021).

26. Deng, J. *et al.* Serological survey of cats in China before and after the pandemic. *Virol. Sin.* **35,** 846–848 (2020).

27. Spada, E. *et al.* A pre- and during pandemic survey of SARS-CoV-2 infection in stray colony and shelter cats from a high endemic area of northern Italy. *Viruses* **13,** 618 (2021).

28. Health, W. O. for A. OIE Technical Factsheet: Infection with SARS-CoV-2 in animals. (2021).

29. Oreshkova, N. *et al.* SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. *Euro Surveill* **25,** 2001005 (2020).

30. Molenaar, R. J. *et al.* Clinical and Pathological Findings in SARS-CoV-2 Disease Outbreaks in Farmed Mink (Neovison vison). *Vet Pathol* **57,** 653–657 (2020).

31. Oude Munnink, B. B. *et al.* Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science (80-.).* **371,** 172–177 (2021).

32. de Rooij, M. M. T. *et al.* Occupational and environmental exposure to SARS-CoV-2 in and around infected mink farms. *medRxiv* **preprint,** (2021).

33. Shuai, L. *et al.* Replication, pathogenicity, and transmission of SARS-CoV-2 in minks. *Natl Sci Rev* (2020). doi:10.1093/nsr/nwaa291

34. Shi, J. *et al.* Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science* **368,** 1016–1020 (2020).

35. Kim, Y.-I. *et al.* Infection and rapid transmission of SARS-CoV-2 in ferrets. *Cell Host Microbe* **27,** 704–709 (2020).

36. Richard, M. *et al.* SARS-CoV-2 is transmitted via contact and via the air between ferrets. *Nat. Commun.* **11,** (2020).

37. Schlottau, K. *et al.* SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *The Lancet Microbe* E218–E225 (2020). doi:https://doi.org/10.1016/S2666-5247(20)30089-6

38. Kutter, J. S. *et al.* SARS-CoV and SARS-CoV-2 are transmitted through the air between ferrets over more than one meter distance. *Nat. Commun.* **12,** 1653 (2021).

39. Bosco-Lauth, A. M. *et al.* Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. *Proc. Natl. Acad. Sci. U. S. A.* **117,** 26382–26388 (2020).

40. Gaudreault, N. N. *et al.* SARS-CoV-2 infection, disease and transmission in domestic cats. *Emerg Microbes Infect* **9,** 2322–2332 (2020).

41. Halfmann, P. J. *et al.* Transmission of SARS-CoV-2 in domestic cats. *N Engl J Med* **383,** 592–594 (2020).

42. Hamer, S. A. *et al.* SARS-CoV-2 infections and viral isolations among serially tested cats and dogs in households with infected owners in Texas, USA. *Viruses* **13,** 938 (2021).

43. Barrs, V. R. *et al.* SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. *Emerg. Infect. Dis.* **26,** 3071–3074 (2020).

44. Hosie, M. J. *et al.* Respiratory disease in cats associated with human-to-cat transmission of SARS-CoV-2 in the UK. *bioRxiv* **preprint,** (2020).

45. Musso, N. *et al.* New SARS-CoV-2 infection detected in an Italian pet cat by RT-qPCR from deep pharyngeal swab. *Pathogens* **9,** 746 (2020).

46. McAloose, D. *et al.* From People to Panthera: Natural SARS-CoV-2 Infection in Tigers and Lions at the Bronx Zoo. *Host-Microbe Biol.* **11,** e02220-20 (2020).

47. Bartlett, S. L. *et al.* SARS-CoV-2 Infection And Longitudinal Fecal Screening In Malayan Tigers (Panthera tigris jacksoni), Amur Tigers (Panthera tigris altaica), And African Lions (Panthera leo krugeri) At The Bronx Zoo, New York, USA. *J. Zoo Wildl. Med.* **51,** 733–744 (2021).

48. Freuling, C. M. *et al.* Susceptibility of Raccoon Dogs for Experimental SARS-CoV-2 Infection. *Emerg. Infect. Dis.* **26,** 2982–2985 (2020).

49. Bosco-Lauth, A. M. *et al.* Survey of peridomestic mammal susceptibility to SARS-CoV-2 infection. *bioRxiv* (2021).

50. Francisco, R. *et al.* Experimental susceptibility of North American raccoons (Procyon lotor) and striped skunks (Mephitis mephitis) to SARS-CoV-2. *bioRxiv* (2021).

51. Griffin, B. D. *et al.* North American deer mice are susceptible to SARS-CoV-2. *bioRxiv* **preprint,** (2020).

52. Fagre, A. *et al.* SARS-CoV-2 infection, neuropathogenesis and transmission among deer mice: Implications for reverse zoonosis to New World rodents. *bioRxiv* **preprint,** (2020).

53. Bertzbach, L. D. *et al.* SARS-CoV-2 infection of Chinese hamsters (Cricetulus griseus) reproduces COVID-19 pneumonia in a well-established small animal model. *Transbound Emerg Dis* **68,** 1075–1079 (2021).

54. Chan, J. F.-W. *et al.* Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin Infect Dis* **71,** 2428–2446 (2020).

55. Sia, S. F. *et al.* Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* **583,** 834–838 (2020).

56. Bao, L. *et al.* The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature* 830–833 (583). doi:https://doi.org/10.1038/s41586-020-2312-y

57. Ulrich, L. *et al.* Experimental SARS-CoV-2 Infection of Bank Voles. *bioRxiv* **preprint,** (2020).

58. Yao, W. *et al.* Circulating SARS-CoV-2 variants B.1.1.7, 501Y.V2, and P.1 have gained ability to utilize rat and mouse Ace2 and altered in vitro sensitivity to neutralizing antibodies and ACE2-Ig. *bioRxiv* **preprint,** (2021).

59. Mykytyn, A. Z. *et al.* Susceptibility of rabbits to SARS-CoV-2. *Emerg Microbes Infect* **10,** 1–7 (2021).

60. Palmer, M. V *et al.* Susceptibility of white-tailed deer (Odocoileus virginianus) to SARS-CoV-2. *J Virol* **95,** e00083-21 (2021).

61. Hall, J. S. *et al.* Experimental challenge of a North American bat species, big brown bat (Eptesicus fuscus), with SARS-CoV-2. *Transbound Emerg Dis* 1–10 (2020). doi:10.1111/tbed.13949

62. Meekins, D. A. *et al.* Susceptibility of swine cells and domestic pigs to SARS-CoV-2. *bioRxiv* **preprint,** (2020).

63. Pickering, B. *et al.* Susceptibility of domestic swine to experimental infection with severe acute respiratory syndrome Ccronavirus 2. *Emerg. Infect. Dis.* **27,** 104–112 (2021).

64. Vergara-Alert, J. *et al.* Piglets inoculated by different routes are not susceptible to SARS-CoV-2, but those inoculated parenterally were immunized against the virus. *Transbound Emerg Dis* (2020). doi:10.1111/tbed.13861

65. Ulrich, L., Wernike, K., Hoffmann, D., Mettenleiter, T. C. & Beer, M. Experimental infection of cattle with SARS-CoV-2. *Emerg. Infect. Dis.* **12,** 2979–2981 (2020).

66. Falkenberg, S. *et al.* Experimental Inoculation of Young Calves with SARS-CoV-2. *Viruses* **13,** 441 (2021).

67. Suarez, D. L. *et al.* Lack of susceptibility to SARS-CoV-2 and MERS-CoV in poultry. *Emerg. Infect. Dis.* **12,** 3071–3076 (2020).

68. Berhane, Y., Suderman, M., Babiuk, S. & Pickering, B. Susceptibility of turkeys, chickens and chicken embryos to SARS-CoV-2. *Transbound Emerg Dis* (2020). doi:https://doi.org/10.1111/tbed.13970

69. Legagneux, P., Lemire, M. & Wong, G. No COVID-19 in snow geese, concluses Université Laval study. (2020). Available at: https://sentinellenord.ulaval.ca/en/news/no-covid-19-snow-geese-concludes-universite-laval-study. (Accessed: 29th June 2021)

70. Xu, L. *et al.* COVID-19-like symptoms observed in the Chinese tree shrews infected with SARS-CoV-2. *Zool Res* **41,** 517–526 (2020).

71. Zhao, Y. *et al.* Susceptibility of tree shrew to SARS-CoV-2 infection. *Sci. Rep.* **10,** (2020).

72. Global, S. D. Z. Gorilla troop at the San Diego Zoo Safari Park test positive for COVID-19. (2021). Available at: https://sandiegozoowildlifealliance.org/pressroom/news-releases/gorilla-troop-san-diego-zoo-safari-park-test-positive-covid-19. (Accessed: 28th June 2021)

73. Lu, S. *et al.* Comparison of SARS-CoV-2 infections among 3 species of non-human primates. *bioRxiv* **preprint,** (2020).

74. Shan, C. *et al.* Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in Rhesus macaques. *Cell Res.* **30,** 670–677 (2020).

75. Rockx, B. *et al.* Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science (80-.).* **368,** 1012–1015 (2020).

76. Munster, V. J. *et al.* Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. *Nature* **585,** 268–272 (2020).

77. Deng, W. *et al.* Ocular conjunctival inoculation of SARS-CoV-2 can cause mild COVID-19 in Rhesus macaques. *Nat. Commun.* **11,** (2020).

78. Singh, D. K. *et al.* Responses to acute infection with SARS-CoV-2 in the lungs of rhesus macaques, baboons and marmosets. *Nat. Microbiol.* **6,** 73–86 (2021).

79. Cross, R. W. *et al.* Intranasal exposure of African green monkeys to SARS-CoV-2 results in acute phase pneumonia with shedding and lung injury still present in the early convalescence phase. *Virol. J.* **17,** (2020).

80. Animals and COVID-19. (2021). Available at: https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/prevention-risks/animals-covid-19.html. (Accessed: 26th July 2021)

81. Charbonneau, R., Niel, L., Olfert, E., von Keyserlingk, M. & Griffin, G. *CCAC guidelines on: euthanasia of animals used in science*. (2010).

82. Leary, S. *et al. AVMA guidelines for the euthanasia of animals: 2020 edition*. (2020).

83. Tamlin, A. L., Bowman, J. & Hackett, D. F. Separating wild from domestic American mink based on skull morphometrics. *Wildlife Biol.* **15,** 266–277 (2009).

84. Trapping Regulations. *Fur Institute of Canada* (2019). Available at: https://fur.ca/fur-trapping/trapping-regulations/. (Accessed: 30th June 2021)

85. *Agreement on international humane trapping standards between the European Community, Canada, and the Russian Federation*. (1997).

86. Austin-Smith, P. *et al. Canadian Council on Animal Care guidelines on: the care and use of wildlife*. (2003).

87. Austin-Smith, P. *et al. Guidelines on: The care and use of wildlife*. (2003).

88. Otis, D. L., Burnham, K. P., White, G. C. & Anderson, D. R. Statistical inference from capture data on closed animal populations. *Wildl. Monogr.* **62,** 3–135 (1978).

89. Sun, C. C., Fuller, A. K. & Royle, J. A. Trap configuration and spacing influences parameter estimates in spatial capture-recapture models. *PLoS One* **9,** e88025 (2014).

90. *Canadian Wildlife Health Cooperative Shipping & Handling Instructions*. (2015).

91. Canadian Association of Zoo and Wildlife Veterinarians. (2020). Available at: https://cazwv.org/wp/. (Accessed: 26th July 2021)

92. Canada, T. Temporary certificate TU 0764.1 - Test samples for COVID-19. (2021). Available at: https://tc.canada.ca/en/dangerous-goods/temporary-certificates/temporary-certificate-tu-07641-test-samples-covid-19. (Accessed: 29th June 2021)

93. Canada, G. of. Biosafety advisory: SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus 2). (2020). Available at: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/biosafety-directives-advisories-notifications/novel-coronavirus-january-27.html#a1. (Accessed: 30th June 2021)

94. Corman, V. M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* **25,** 2000045 (2020).

95. Research use only 2019-novel coronavirus (2019-nCov) real-time RT-PCR primers and probes. *Centers for Disease Control and Prevention* (2020). Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html. (Accessed: 26th July 2021)

96. *Interim guidance for laboratories testing animals for SARS-CoV-2*.

97. Xiu, L. *et al.* A RT-PCR assay for the detection of coronaviruses from four genera. *J. Clin. Virol.* **128,** 104391 (2020).

List of Annexes

Annex I. Flow chart for SARS-CoV-2 surveillance of wildlife in Canada

Annex II. Contacts for the Chief Veterinary Officer in each province/territory

Annex III. Contacts leading targeted wildlife surveillance for SARS-CoV-2 in each province/territory

Annex IV. Laboratory contact information for submission of wildlife samples for:

- SARS-CoV-2 RT-PCR testing of samples obtained through targeted surveillance
- Serological testing of samples obtained through targeted or opportunistic surveillance
- Pan-coronavirus PCR testing, to test samples for the presence of all coronaviruses, for samples collected through targeted or opportunistic surveillance
- Genetic testing of mink samples

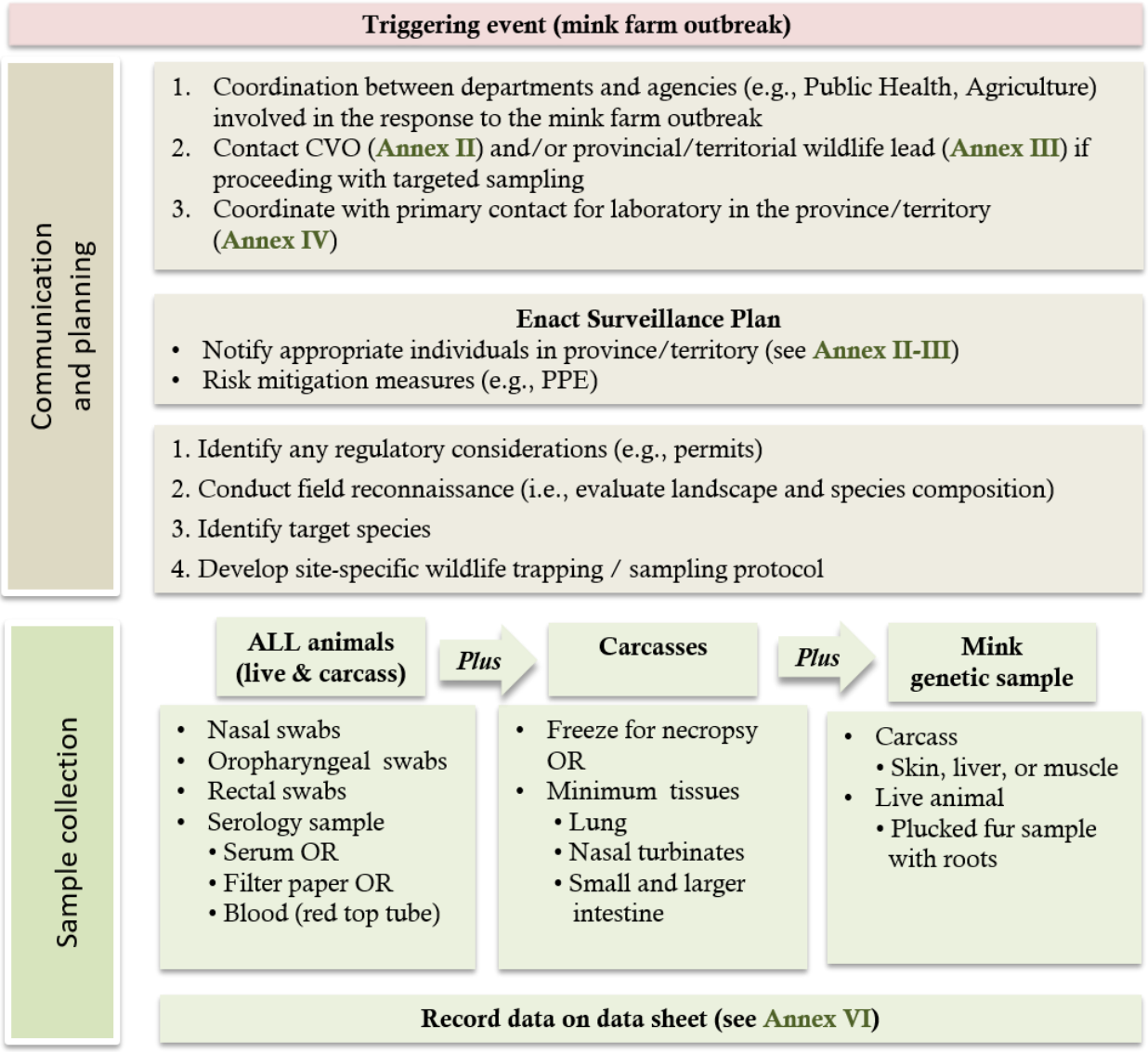
Annex V. Flow chart for testing for SARS CoV-2 in wildlife

Annex VI. Sample collection data sheets:

- For apparently healthy wildlife animals not suspected to have SARS-CoV-2 (live/dead):
  - Sample collection data sheet for targeted SARS-CoV-2 surveillance in wildlife targeted SARS-CoV-2 surveillance in wildlife
- For wildlife suspected to have SARS-CoV-2:
  - Full necropsy procedure and sample inventory check list for SARS-CoV-2 surveillance

Annex VII. UN3373 for Biological Substance Category B label template

Annex I. Flow chart for SARS-CoV-2 surveillance of wildlife in Canada



Sample handling and transport	**Ship samples to participating diagnostic lab(s) for PCR, serology and necropsy (see Annex IV)**	
	For immediate submission: • Store all samples on ice packs or in refrigerator • Ship on ice (see **Section 7**)	**For batch submission:** • Freeze samples ASAP and keep frozen • Ship on solid ice packs or on dry ice (see **Section 7**)
Confirmation	**Non-negative PCR results:** • See **Annex V** for confirming and communicating non-negative PCR results • Send duplicate samples and cDNA to NCFAD (Winnipeg) for confirmation • If feasible: necropsy	**Negative PCR results:** • Carcass and samples may be disposed of according to provincial / territorial guidelines

Annex II. Contacts for the Chief Veterinary Officer in each province/territory

Province	Name	Email	Phone number
British Columbia	**Dr. Brian Radke** Public Health Veterinarian, Livestock Health Management and Regulatory Unit, Government of British Columbia	brian.radke@gov.bc.ca	778-666-0544
Alberta	**Dr. Keith Lehman** Chief Provincial Veterinarian, Office of the Chief Provincial Veterinarian, Government of Alberta	keith.lehman@gov.ab.ca	708-427-6406
Saskatchewan	**Dr. Stephanie Smith** Chief Veterinary Officer, Veterinary Unit, Ministry of Agriculture, Government of Saskatchewan	stephanie.smith@gov.sk.ca	306-527-3350

Manitoba	**Dr. Dale Douma** Public Health Epidemiologist, Animal Health and Welfare Branch, Manitoba Agriculture & Resource Development, Government of Manitoba	dale.douma@gov.mb.ca	204-945-8011
Ontario	**Dr. Cathy Furness** Chief Veterinarian, Animal Health and Welfare Branch/ Office of the Chief Veterinarian for Ontario, Government of Ontario	ag.info.omafra@ontario.ca cathy.furness@ontario.ca	1-877-424-1300
Quebec	**Dr. Hélène Trépanier** Médecin vétérinaire en chef, Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, Gouvernement du Québec	Contact through link at the end of the annex*	1-844-ANIMAUX 1-844 264-6289

New Brunswick	**Dr. Nicole Wanamaker** Department of Agriculture, Aquaculture and Fisheries, Government of New Brunswick	nicole.wanamaker@gnb.ca	506-433-0493
Nova Scotia	**Dr. Wilma Schenkels** Department of Agriculture, Government of Nova Scotia	dr.wilma.schenkels@novascotia.ca	902-890-2941
Prince Edward Island	**Dr. Jill Wood** Provincial Veterinarian, Department of Agriculture and Land, Government of Prince Edward Island	jswood@gov.pe.ca	902-370-4923
Newfoundland and Labrador	**Dr. Beverly Dawe** Chief Veterinary, Officer and Divisional Director Animal Health Division, Government of Newfoundland and Labrador	beverlydawe@gov.nl.ca	709-637-2042 or 709-639-2121

Yukon	**Dr. Mary VanderKop** Chief Veterinary Officer, Animal Health Unit Department of the Environment, Government of Yukon	mary.vanderkop@yukon.ca	867-667-5600
Northwest Territories	**Dr. Naima Jutha** Wildlife Veterinarian, Chief Veterinary Officer Wildlife & Fish Division Department Environment & Natural Resources, Government of the Northwest Territories	naima_jutha@gov.nt.ca	867-767-9237 ext. 53232
Nunavut	**Dr. Wanda Joy** Environmental Health Consultant, Department of Health, Government of Nunavut	wjoy@gov.nu.ca	867-222-2373

*https://www.mapaq.gouv.qc.ca/fr/Productions/santeanimale/centrale/Pages/Fichedesignalementsanteanimale.aspx

Annex III. Contacts leading targeted wildlife surveillance for SARS-CoV-2 in each province/territory

Province	Name	Email	Phone number
British Columbia	**Dr. Caeley Thacker** Wildlife Veterinarian, Wildlife and Habitat Branch, Wildlife Management, Government of British Columbia	caeley.thacker@gov.bc.ca	250-751-3219
Alberta	**Dr. Margo Pybus** Provincial Wildlife Disease Specialist, Species at Risk and Stewardship, Environment and Parks, Government of Alberta	margo.pybus@gov.ab.ca	780-427-3462
Saskatchewan	**Dr. Iga Stasiak** Provincial Wildlife, Health Specialist Strategic Conservation Ministry of Environment, Government of Saskatchewan	iga.stasiak@gov.sk.ca	306-728-7713

Manitoba	**Dr. Richard Davis** Wildlife Health Biologist, Manitoba Agriculture and Resource Development Ecosystem Management Division, Wildlife, Fisheries and Resource Enforcement Branch Game, Fur and Wildlife Science, Government of Manitoba	richard.davis@gov.mb.ca	204-648-5320
Ontario	**Dr. Jeff Bowman** Research Scientist, Ministry of Northern Development, Mines, Natural Resources and Forestry, Government of Ontario	jeff.bowman@ontario.ca	705-875-1748
Quebec	**Dr. Ariane Massé** Biologiste, Ministère des Forêts, de la Faune et des Parcs, Gouvernement du Québec	santedelafaune@mffp.gouv.qc.ca ariane.masse@mffp.gouv.qc.ca	1-877-346-6763

New Brunswick	**Dr. Kevin Case** Acting Director, Fish and Wildlife, Department of Natural Resources and Energy Development, Government of New Brunswick	kevin.case@gnb.ca	506-429-3021
Nova Scotia	**Dr. Glen Parsons** Manager, Sustainable Wildlife use, Department of Lands and Forestry, Government of Nova Scotia	glen.parsons@novascotia.ca	902-679-6091
Prince Edward Island	**Dr. Jill Wood** Provincial Veterinarian, Department of Agriculture and Land, Government of Prince Edward Island	jswood@gov.pe.ca	902-370-4923
Newfoundland and Labrador	**Dr. Wayne Barney** Senior Wildlife Biologist, Habitat, Game and Fur Program, Wildlife Division Department of Fisheries, Forestry and Agriculture, Government of Newfoundland and Labrador	waynebarney@gov.nl.ca	709-637-2014

Yukon	**Dr. Kristenn Magnusson** Program Veterinarian, Animal Health Unit, Department of the Environment, Government of Yukon	kristenn.magnusson@yukon.ca	867-667-5600 or 867-667-8663
Northwest Territories	**Dr. Naima Jutha** Wildlife Veterinarian, Chief Veterinary Officer, Wildlife & Fish Division Department Environment & Natural Resources, Government of the Northwest Territories	naima_jutha@gov.nt.ca	867-767-9237 ext. 53232
Nunavut	**Dr. Wanda Joy** Environmental Health Consultant, Department of Health, Government of Nunavut	wjoy@gov.nu.ca	867-222-2373

Annex IV. Laboratory contact information for submission of wildlife samples for:

SARS-CoV-2 RT-PCR testing of samples obtained through targeted surveillance

Lab	Address	Email	Phone number
Animal Health Centre, BC Ministry of Agriculture	1767 Angus Campbell Road Abbotsford, B.C. V3G 2M3	PAHB@gov.bc.ca	604-556-3003 1-800-661-9903
Prairie Diagnostic Services	52 Campus Drive Saskatoon, SK S7N 5B4	pds.info@usask.ca	306-966-7316
Veterinary Diagnostic Services	545 University Crescent Winnipeg, Manitoba R3T 5S6	vetlab@gov.mb.ca	204-945-8220
Animal Health Lab, University of Guelph	Box 3612 Guelph, Ontario N1H 6R8	ahlinfo@uoguelph.ca	519-824-4120 ext. 54530

Laboratoire des maladies infectieuses virales vétérinaires et Laboratoires de diagnostic virologique vétérinaire, de sérologie aviaire, de diagnostic moléculaire, de séquençage à haut débit et de microscopie électronique, Faculté de médecine vétérinaire, Université de Montréal	3200 Sicotte, Bureau 3963 Saint-Hyacinthe, QC J2S 2M2	carl.a.gagnon@umontreal.ca	450-773-8521 # 8681

Serological testing of samples obtained through targeted or opportunistic surveillance

Primary contact	Email	Phone number
Dr. Robbin Lindsay Research Scientist, Zoonotic Diseases and Special Pathogens, National Microbiology Laboratory, Public Health Agency of Canada	robbin.lindsay@canada.ca	Office: 204-789-6060 Cell: 204-228-1088

Dr. Brad Pickering Research Scientist, Special Pathogens Unit, Canadian Food Inspection Agency	bradley.pickering@canada.ca	204-789-7620
Dr. Scott Weese Professor, Ontario Veterinary College, University of Guelph	jsweese@uoguelph.ca	519-824-4120 ext. 54064

A serological test are also being developed at the University of Saskatchewan, Western College of Veterinary Medicine. Contact the following individuals for more information.

Primary contact	Email	Phone number
Dr. Vikram Misra Professor, Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan	vikram.misra@ec.gc.ca	306-966-7218
Dr. Catherine Soos Research Scientist, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, Saskatoon, SK Adjunct Professor, Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan	catherine.soos@ec.gc.ca	306-975-5357
Dr. Emily Jenkins Professor and Department Head (Acting) Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan	emily.jenkins@usask.ca	306-966-2569

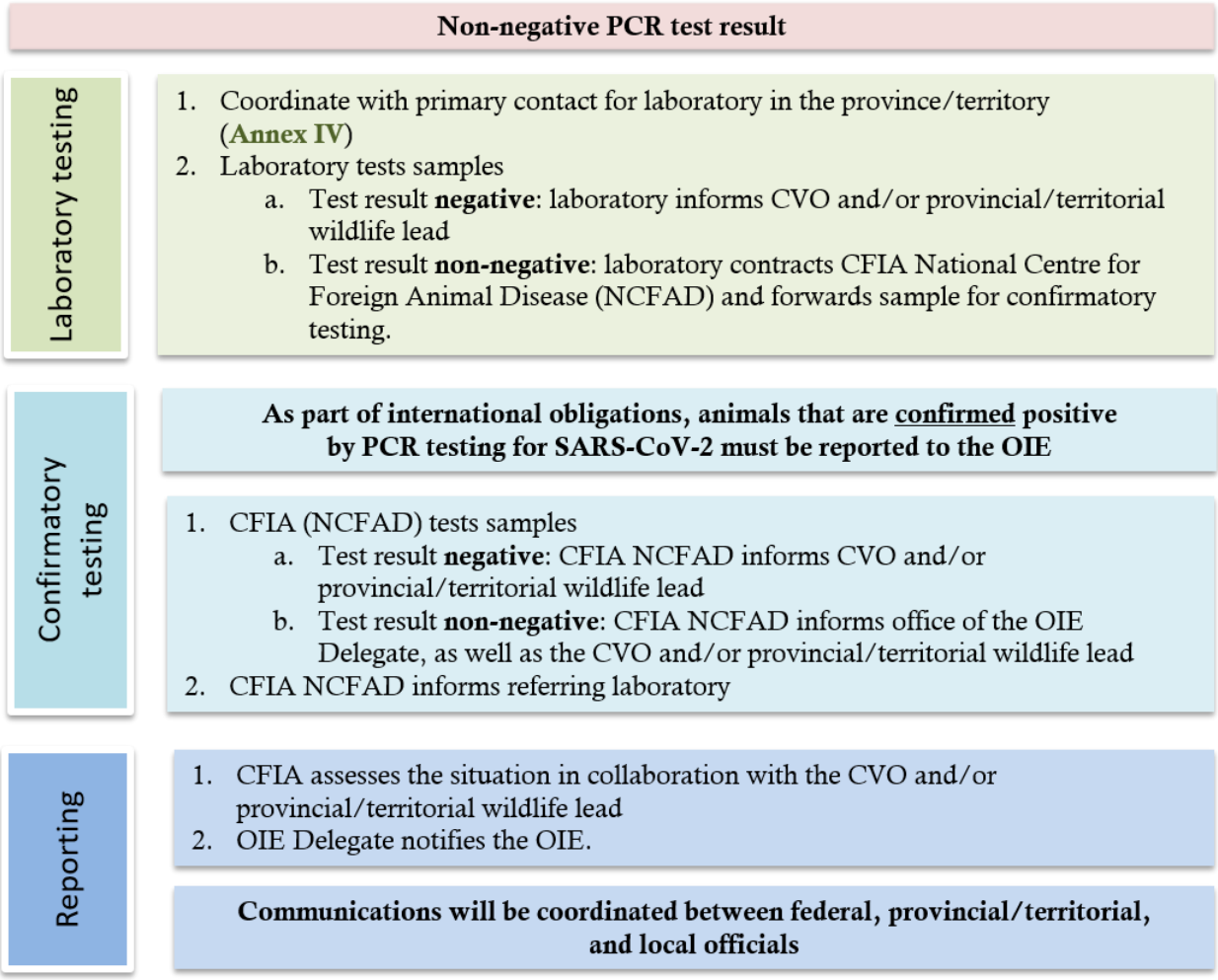
Pan-coronavirus PCR-test samples for the presence of all coronaviruses, for samples collected through targeted or opportunistic surveillance

Primary contact	Email	Phone number
Dr. Catherine Soos Research Scientist, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, Saskatoon, SK	catherine.soos@ec.gc.ca	306-975-5357
Dr. Emily Jenkins Professor and Department Head (Acting) Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan	emily.jenkins@usask.ca	306-966-2569
Dr. Samira Mubareka Scientist Sunnybrook Health Sciences Centre	samira.mubareka@sunnybrook.ca	416-480-4823

Genetic sampling of mink samples

Primary contact	Email	Phone number
Dr. Jeff Bowman Research Scientist, Ministry of Northern Development, Mines, Natural Resources and Forestry, Government of Ontario	jeff.bowman@ontario.ca	705-875-1748

Annex V. Flow chart for confirming and communicating a non-negative PCR test result for SARS CoV-2 in wildlife



For contacts and more information on the confirmation of non-negative samples and the reporting process please refer to the CFIA Interim Guidance for Laboratories Testing Animals for SARS-CoV-2

Annex VI. Sample collection data sheets

The following sample collection data sheets are available on the following pages:

For apparently healthy wildlife animals not suspected to have SARS-CoV-2 (live/dead):

Sample collection data sheet for targeted SARS-CoV-2 surveillance in wildlife targeted SARS-CoV-2 surveillance in wildlife

For wildlife suspected to have SARS-CoV-2:

Full necropsy procedure and sample inventory check list for SARS-CoV-2 surveillance

Sample Collection Datasheet for Coronavirus Surveillance in Wild Mammals

Date of collection:_____ **Date of necropsy:**_____

Province:_____ **Location (City/Town/Latitude/Longitude):**_____

Collected by: _____ **Sampled by:**_____

Specimen ID: _____ **Species:**_____ **Sex: ☐M ☐F Age:**_____

Samples collected. Please indicate what has been collected, and ensure all samples are labelled with the <u>Specimen ID</u> and <u>sample type</u>.

Swab checklist (for PCR)	**Samples for serology:**	**Additional tissues:**	
Please collect <u>all three, in duplicate</u>: ☐ Nasal* ☐ Oropharyngeal* ☐ Rectal **Vial media:** ☐ VTM ☐ Lysis buffer **If preferred, the same swab can be used to sample nasal and oropharyngeal cavity.*	**Blood for serology (select <u>one</u>):** ☐ Serum ☐ Heart blood ☐ Blood on filter paper, 2 strips	**Freeze back carcass?** ☐ Yes ☐ No **If not, please collect tissues for subsequent testing (check all that apply):** ☐ Lung (~5g) ☐ Nasal turbinates ☐ Small intestine (~5g) ☐ Large intestine (~5g) ☐ Retropharyngeal lymph node (e.g., from deer heads) ☐ Other_____	**Tissue for DNA analysis (<u>mink only</u>)** ☐ Fur (envelope or ziploc, room temp) **or** ☐ Skin ☐ Muscle ☐ Other_____ **Indicate whether:** ☐ frozen, or ☐ in lysis buffer

Necropsy observations (Check parameters that apply.):

State of carcass:	☐ Whole ☐ Partial ☐ Scavenged ☐ Dried ☐ Decomposed
Internal fat stores:	☐ 0 (depleted) ☐ 1 (slight) ☐ 2(moderate) ☐ 3 (abundant)
Mass / Morphometric measurements:	

Other observations: ***(List any abnormalities, signs of illness, e.g., nasal discharge lung lesions, other lesions, etc.):***	

Live animal observations (if applicable):

General observations:	☐ Appeared healthy ☐ Appeared sick
Clinical signs:	☐ Respiratory signs ☐ Other signs/behavioural abnormalities? (Describe below.)
Other observations:	

ADDITIONAL NOTES (if any):

Necropsy Guidelines and Sample Checklist for Wildlife that are Confirmed or Suspect Positive for SARS-CoV-2

This sample inventory list is intended to aid prosectors who may not routinely perform necropsies on wildlife species. This list is intended to facilitate the collection of an optimum set of samples for the diagnosis and characterization of lesions in wildlife infected with SARS-CoV-2. The list does not replace existing sample collection or submission forms utilized by diagnostic laboratories.

Sample collection and handling for virology, histopathology, and serology should ideally follow protocols required by testing laboratories; it is always recommended to contact the relevant laboratory prior to sample collection to ensure appropriate samples are acquired.

Biosafety: Lab safety guidelines should follow the Biosafety Advisory published by the Public Health Agency of Canada. Necropsy should be performed in a facility that meets the requirements for Containment Level 2 (CL2) as specified by the Canadian Biosafety Standard. See Wildlife Surveillance Guidelines in Response to the Detection of SARS-CoV-2 in Farmed Mink in Canada, Section 8.1 for more details.

If necropsies are being performed on multiple animals, ensure tools and surfaces are thoroughly disinfected between animals to prevent cross-contamination.

Tissue samples should be collected using clean instruments, and samples collected for freezing should be collected early in the necropsy, after opening the body and prior to handling organs. For histology, samples should be no more than one cm thick, but should be large enough to be representative of the organ sampled. Samples for histology should be completely submerged in 10% neutral buffered formalin at a ratio of 1:10 tissue to formalin.

In the checklist below:

SWAB samples should be taken prior to the necropsy (prior to opening the carcass). Swabs from the nasal sinuses, oral cavity, and rectum should be taken, particularly in animals not yet tested for SARS-CoV-2. Tracheal swabs may also be collected if lesions observed.

Fix = Submerge in 10% neutral buffered formalin.

Freeze = Place in a sterile, labeled leak-proof container and refrigerate as soon as possible. If samples are to be retained, freeze at -80 C within 12 hours. If a -80C freezer is not available, place in a regular (-20C) freezer. Duplicate frozen samples are recommended if multiple ancillary tests are expected.

In addition to the recommended samples, additional samples of any tissue that appears grossly abnormal are strongly recommended. Gross photography is also recommended, to document any lesions.

Following the necropsy, carcasses should be disposed of following provincial / territorial guidelines.

NECROPSY SAMPLE CHECKLIST FOR WILDLIFE SUSPECTED OR CONFIRMED TO HAVE SARS-COV-2

Specimen ID: _____ **Species**:_____ **Sex**: ☐M ☐F **Age**:_____

SARS-CoV-2 Status: ☐ Not previously tested ☐ Pending ☐ Neg ☐ Presumptive POS ("Non-neg") ☐ Confirmed POS

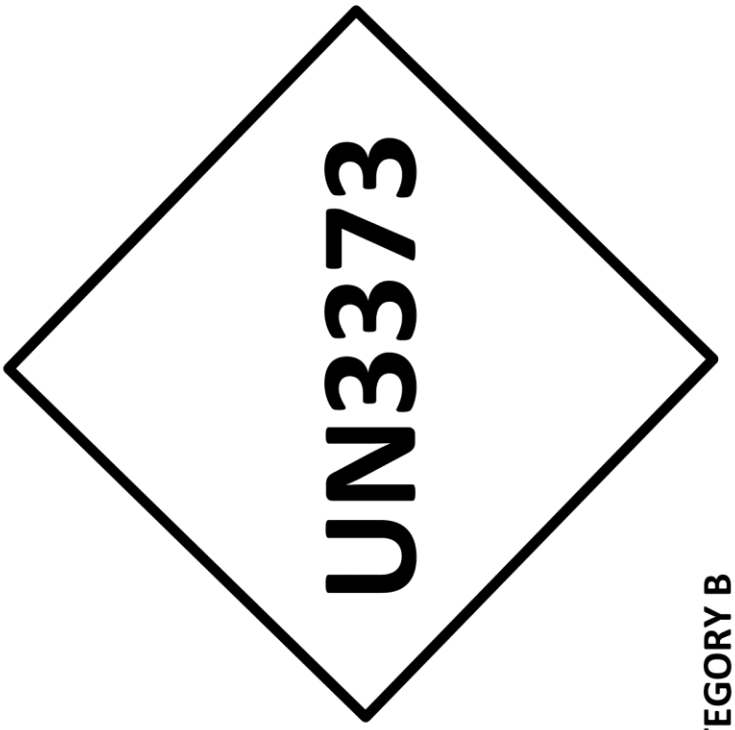
Tested by (lab): _____

Prosector(s):_____ **Date of Necropsy**:_____

SWABS	
☐ Nasal ☐ Tracheal ☐ Oropharyngeal ☐ Rectal	**Media**: ☐ VTM ☐ Lysis ☐ Other: _____

PRIORITY TISSUES			
Fix	**Freeze**	**Tissue**	**Comments/Photo log:**
		Nasal turbinates	
		Trachea	
		Lung (sample L and R sides)	
		Bronchus	
		Mediastinal lymph node (label or leave attached to lung)	
		Heart (fix cross section at apex), and freeze heart blood or blood-soaked filter paper strips (e.g. Nobuto)	
		Liver (fix at least 2 sections)	
		Spleen	
		Kidney (include cortex, medulla, pelvis)	
		Pancreas	
		Stomach	
		Small intestine	
		Large intestine	
		Mesenteric lymph node (leave attached to GI or label)	
		Brain (include cortex, cerebellum, brainstem)	
		Pregnant: Placenta	
		Pregnant: Fetal tissues	
LOWER PRIORITY TISSUES			
		Skin (label site)	
		Salivary gland	
		Other lymph nodes (label)	
		Diaphragm	
		Tongue	
		Urinary bladder	
		Adrenal glands	
		Eye (label L or R)	
		Uterus (fix sections of horns and body)	
		Skeletal muscle	
		Spinal cord	
		Peripheral nerve	
		Thyroid gland	
		Pituitary gland	
		Mammary gland	
		Bone marrow	
		OTHER:	
		OTHER:	

Annex VII. UN3373 for Biological Substance Category B label template



List of contributors

Leads:

- **Catherine Soos**, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, Saskatoon, SK
- **Megan Jones**, Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island / Canadian Wildlife Health Cooperative: Atlantic Region

Additional contributors:

- **Emilie Bouchard**, Wildlife Management and Regulatory Affairs Division, Canadian Wildlife Service, Environment and Climate Change Canada
- **Jeff Bowman**, Ministry of Northern Development, Mines, Natural Resources and Forestry, Government of Ontario / Trent University
- **Laura Bruce**, Ministry of Northern Development, Mines, Natural Resources and Forestry, Government of Ontario
- **Indervesh Dahiya**, Animal Health Directorate, Canadian Food Inspection Agency
- **Farouk El Allaki**, Animal Health Epidemiology and Surveillance Section, Animal Health Science Directorate, Science Branch, Canadian Food Inspection Agency
- **Logan Flockhart**, Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, Publish Health Agency of Canada
- **Maureen Freemark**, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada
- **Marianne Gagnier**, Ministère des Forêts, de la Faune et des Parcs, Gouvernement du Québec
- **Chelsea Himsworth**, Animal Health Center, Government of British Columbia
- **Emily Jenkins**, Western College of Veterinary Medicine, University of Saskatchewan
- **Pratap Kafle**, Western College of Veterinary Medicine, University of Saskatchewan

- **Daniel Leclair**, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada
- **Jamille McLeod**, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada
- **Landon McPhee**, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada
- **Cynthia Pekarik**, Wildlife Management and Regulatory Affairs Division, Canadian Wildlife Service, Environment and Climate Change Canada
- **Julie Pare**, Animal Health Epidemiology and Surveillance Section, Animal Health Science Directorate, Scintce Branch, Canadian Food Inspection Agency
- **Iga Stasiak**, Strategic Conservation Ministry of Environment Government of Saskatchewan
- **Craig Stephen**, School of Population and Public Health, University of British Columbia / Ross University School of Veterinary Medicine
- **Caeley Thacker**, Wildlife and Habitat Branch, Wildlife Management Government of British Columbia
- **Bruce Rodrigues**, Fisheries, Forestry and Agriculture Department, Government of Newfoundland and Labrador
- **Alana Wilcox**, Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada