

## Recommended Protocol for Sampling and Banking Tissues from Wild Pigs

### **Purpose of tissue bank:**

1. To establish a collection of tissues from wild pigs from across Canada that can be used for retrospective disease testing in case of a disease event (to contribute to the epidemiological investigation thereof) OR for future approved targeted research or surveillance projects, including test validation.
2. To establish a system through which tissues samples from wild pigs are routinely collected by laboratory staff and individuals in the field (e.g. hunters) - including the relationships necessary for such collaborations - that can be ramped up in case of a disease event or other need for targeted testing of wild pigs.
3. This protocol is a guideline for hunters, field personnel and laboratory staff that may harvest or receive wild pigs occasionally. It is not intended to replace already existing sampling protocols.

To be clear, the purpose of the tissue bank is NOT directly to detect foreign animal disease nor to demonstrate disease freedom in the wild pig population, but to establish in advance a system/routine for sample collection that can be used or modified to contribute to these goals when the need arises.

**Location of tissue bank:** Tissues can be stored at any provincial or academic laboratory, but will be catalogued centrally using the Canadian Wildlife Health Cooperative (CWHC) WHIP system. Non-CWHC labs can send information on the samples they have stored to the CWHC for inclusion in the inventory. Samples collected that cannot be stored locally may be sent to CWHC (Saskatchewan) for storage. Please contact the CWHC ([national@cwbc-rscf.ca](mailto:national@cwbc-rscf.ca)) for more information. The inventory will be reviewed every two years, and those storing samples will be asked to verify that the samples in the catalog are still available (accounting for use of samples as well as attrition due to prolonged storage, if applicable). Tissue samples remain the property of whatever facility has them in their physical possession unless an alternate agreement is established on a case-by case basis.

**Accessing the tissue bank:** Requests for use of tissues from the bank can be submitted to the CWHC via [national@cwbc-rscf.ca](mailto:national@cwbc-rscf.ca) and will be reviewed by members of the wild pig surveillance subcommittee for approval. The goal of this system is to help coordinate tissue testing between projects and to ensure the catalog stays up-to-date as much as possible.

- CWHC will keep track of any additional lab-specific requirements that need to be fulfilled (e.g. material transfer agreement for samples from Alberta).

**Limitations of the tissue bank:** While the goal of this protocol is to provide some level of standardization for sample collection and storage, by necessity samples will be collected by a wide variety of people under varying circumstances, and the ability to transport and store samples under optimal conditions in some cases may be limited. Every effort will be made to note these factors in the electronic inventory in order to assist those who may wish to access the tissue samples with selection of those that will best suit their specific purposes and are most likely to yield diagnostic results. Potential users of the bank should assess suitability for their purposes prior to requesting samples.

### **Storage recommendations for each sample type:**

- All tissue samples collected should ideally be frozen at a maximum temperature of -80°C for long term storage. Serum samples can be stored indefinitely at -20°C.
- Short-term storage (less than one month) of tissue samples at -20°C is acceptable, but the duration of storage at any temperature above -80°C, as well as any freeze-thaw cycles the sample may have undergone (e.g. during shipping from one location to another) should be included in the sample information in the catalog. This will assist with assessment of sample quality.

**Sample collection methods:** Sample collection is at the discretion of the individual who finds and/or ultimately deals with the carcass. Sample collection supplies will not be routinely provided by CWHC unless a specific arrangement is established on a case-by-case basis. One of the following three sample collection methods should be specified on the data collection for each sample collected, in order to assist with assessment of sample quality.

- *Field collection*: tissues collected outdoors with many opportunities for microbial contamination of samples.
- *Clean technique*: tissues collected with effort to reduce microbial contamination by use of clean tools, prompt individual bagging of samples, and some environmental control.
- *Sterile technique*: tissues collected and bagged with very low risk of microbial contamination (e.g. controlled environment or laboratory setting using sterile tools).

**NOTE:**

- The wild pig sampling protocols provided herein are based on the assumption that wild pigs in Canada are at low risk for foreign animal diseases, and therefore do not address issues of carcass disposal and cleaning and disinfection protocols for specific pathogens. Should this situation change, supplementary guidance/protocols will need to be provided to anyone collecting tissue samples from wild pig carcasses, particularly in the field.
- **If in any case a reportable disease is suspected in a wild pig, the appropriate federal and provincial authorities must be notified immediately** (i.e. before leaving the site where the pig was killed or the carcass was found, if possible).

**Wild Pig Sampling Datasheet – Field**

**Wild Pig ID#:** \_\_\_\_\_

Wild Pig ID # is the year / month/ day the sample was collected, followed by the two letter abbreviation of the province in which the pig was found plus a two letter code of the collector’s initials and then an individual pig number based on the number of pigs sampled if they were part of a group. For example, the second of three pigs in a group sampled in Saskatchewan by R. Smith on 08-Feb-2021 would be numbered “210208-SKRS-02”. **Please include contact information at the bottom of the page.**

The ID number should be clearly written on each sample container / bag containing tissues from that individual pig, along with the tissue type (e.g. “liver”).

**Wild Pig Information**

Location found (GPS coordinates or nearest crossroad/municipality): \_\_\_\_\_

Apparent species (circle one): wild boar / hybrid / domestic pig / pot-bellied pig / other

Sex (circle one): male – castrated Y/N / Female – pregnant Y/N / Unsure

Estimated age (circle one): piglet / juvenile / adult / unsure

Apparent cause of death (circle one): shot / predation / other injury / unknown / other

Please add any additional pertinent information (eg. Number/weight/sex of fetuses) to the bottom or back of this page.

**Tissues to collect**

Please attempt to collect **as many of the following samples as possible** from each carcass. Please place each sample type in an individual baggie or container and label as per instructions above. Duplicate samples are welcome if possible. The samples from each pig can then be placed in a larger bag labelled with the wild pig ID# for transport and temporary storage. **Alternatively, the head, gut pile and long bone can be bagged and delivered to a veterinary pathology lab for sampling.**

Tissue Type	Amount	Collected	Duplicate collected
Feces / rectal content	50 mL	<input type="checkbox"/>	<input type="checkbox"/>
Lung	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Liver	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Spleen	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Tongue	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Diaphragm	whole or half	<input type="checkbox"/>	<input type="checkbox"/>
Skeletal muscle from leg	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Ear	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Serum	5-10 mL	<input type="checkbox"/>	<input type="checkbox"/>
Long bone (if carcass too decomposed)	whole	<input type="checkbox"/>	<input type="checkbox"/>
If identifiable:			
Tonsil	whole (lt & rt)	<input type="checkbox"/>	<input type="checkbox"/>
Sub-mandibular lymph node	whole (lt & rt)	<input type="checkbox"/>	<input type="checkbox"/>
Superficial inguinal lymph node	whole (lt & rt)	<input type="checkbox"/>	<input type="checkbox"/>

Please contact the CWHC ([national@cwbc-rscf.ca](mailto:national@cwbc-rscf.ca)) for additional information, questions, guidance on sampling techniques (if needed) and to submit this form for inclusion of the samples in the tissue bank inventory. Also please let us know how we can improve this form to make it easier for you to use!

## Wild Pig Sampling Datasheet – Laboratory

**Wild Pig ID#:** \_\_\_\_\_

Wild Pig ID # is the year / month/ day the sample was collected, followed by the two letter abbreviation of the province in which the pig was found plus a two letter code of the collector’s initials and then an individual pig number based on the number of pigs sampled if they were part of a group. For example, the second of three pigs in a group sampled in Saskatchewan by R. Smith on 08-Feb-2021 would be numbered “210208-SKRS-02”.

The ID number should be clearly written on each sample container / bag containing tissues from that individual pig, along with the tissue type (e.g. “liver”).

### Wild Pig Information

Location found (GPS coordinates or nearest crossroad/municipality): \_\_\_\_\_

Apparent species (circle one): wild boar / hybrid / domestic pig / pot-bellied pig / other

Sex (circle one): male / castrated male / pregnant female / non-pregnant female / unsure

Estimated age (circle one): nursing / weanling / juvenile / mature / aged / unsure

Apparent cause of death (circle one): shot / predation / other injury / unsure / other

Please add any additional pertinent information to the bottom or back of this page.

### Tissues to collect

Please attempt to collect as many of the following samples as possible from each carcass. Please place each sample type in an individual baggie or container and label as per instructions above. Duplicate samples are welcome if possible. Sampling method: F = field, C = clean, S = sterile.

Tissue Type	Amount	Collected	Duplicate collected	Method (circle one)
Feces / rectal content	50 mL	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Lung	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Liver	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Spleen	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Tongue	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Diaphragm	whole or half	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Skeletal muscle from leg	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Ear	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Tonsil	whole (lt & rt)	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Superficial inguinal lymph node	whole (lt & rt)	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Sub-mandibular lymph node	whole (lt & rt)	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Mesenteric lymph node	whole (lt & rt)	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
		<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Testicles	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Uterine wall	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Brain	Half	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Serum (if possible)	5-10 mL	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Nasal swab (deep)	1 swab	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S
Long bone (if carcass too decomposed)*	whole	<input type="checkbox"/>	<input type="checkbox"/>	F / C / S

\* The long bone does not require -80C storage.

Please contact the CWHC ([national@cwbc-rscf.ca](mailto:national@cwbc-rscf.ca)) for additional information, questions, guidance on sampling techniques (if needed) and to submit this form for inclusion of the samples in the tissue bank inventory. Also please let us know how we can improve this form to make it easier for you to use!

## Wild Pig Sampling Datasheet –Hunter

**Wild Pig ID#:** \_\_\_\_\_

Wild Pig ID # is the year / month/ day the sample was collected, followed by the two letter abbreviation of the province in which the pig was found plus a two letter code of the collector's initials and then an individual pig number based on the number of pigs sampled if they were part of a group. For example, the second of three pigs in a group sampled in Saskatchewan by R. Smith on 08-Feb-2021 would be numbered "210208-SKRS-02". **Please include contact information at the bottom of the page.**

The ID number should be clearly written on each sample container / bag containing tissues from that individual pig, along with the tissue type (e.g. "liver").

### Wild Pig Information

Location found (GPS coordinates preferred):  
\_\_\_\_\_

Apparent species (circle one): wild boar / hybrid / domestic pig / pot-bellied pig / other

Sex (circle one): male – castrated Y/N / Female – pregnant Y/N / Unsure

Estimated age (circle one): piglet / juvenile / adult / unsure

Apparent cause of death (circle one): shot / predation / other injury / unknown /

Please add any additional pertinent information (eg. Number/weight/sex of fetuses) to the bottom or back of this page.

### Tissues to collect

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Tissue Type	Amount	Collected	Duplicate collected
Feces / rectal content	50 mL	<input type="checkbox"/>	<input type="checkbox"/>
Lung	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Liver	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Spleen	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Tongue	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Diaphragm	whole of half	<input type="checkbox"/>	<input type="checkbox"/>
Skeletal muscle from leg	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Ear	2 inch square	<input type="checkbox"/>	<input type="checkbox"/>
Long bone (if carcass too decomposed)	whole	<input type="checkbox"/>	<input type="checkbox"/>
If identifiable:			
Tonsil	whole (lt&rt)	<input type="checkbox"/>	<input type="checkbox"/>
Sub-mandibular lymph node	whole (lt&rt)	<input type="checkbox"/>	<input type="checkbox"/>
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