

ANNEX 2 – Determination of Population Numbers, Sample Size numbers and Sample Collection

Instructions

The number of populations within any type of culture system will depend on the degree of separation of all animals within the rearing or holding units in the premises. If there is no separation of animals within the premises then sampling must be conducted at the premises level (the premises/all holding units are considered one discrete population). If the premises contain many discrete populations then each sub population should be individually sampled.

1. Determine the number of populations

To determine the number of populations in premises, the following steps must:

1.1 Determine if the water supply is pathogen free.

a) Is the water supply a “fish free” ground water source?

If yes, Go to **1.2**.

Otherwise,

If water treatment being applied at the premises level then consider all the aquatic animals in all holding units as **one population**.

If water treatment is applied at the holding unit level (i.e. tank or pond or raceway) then each holding unit is considered a **discrete population**.

***NOTE:** If water treatment is being used, biosecurity measures to prevent introduction of pathogens between holding units is required. Water treatment must be adequate to eliminate pathogens. Some accepted methods include chlorination, ozone and UV. For parasites, physical filtration (sand filters to 0.5 microns) may also be adequate.

1.2 Determine the water flow between holding units:

If water is shared between holding units such that pathogens of concern can also be shared then all animals in those holding units are considered **one population**.

If water is not shared then each holding unit is a **discrete population**.

2. Calculating sample size

Table 1 is used to identify the sample size required, for each population, to be confident that, if the disease is present, it is present at a level lower than that specified minimum expected (threshold) prevalence. For the purposes of exporting to Canada the threshold prevalence is 2%.

- i. Identify the population or populations for sampling outlined in Step 1.
- ii. Determine the number of holding units within the/those population(s).
- iii. Use Table 1 to determine the sample size.

NB: Where the population size is less than 1000 animals lethal sampling may not be acceptable as sample sizes are too large.

Table 1: Sample Size for Healthy Populations as Determined by Population Size, Threshold Prevalence and a 95% Confidence of Detecting Disease.

Population size ¹	Threshold prevalence (%)	Confidence (%)	Sample Size ²	
			Statistical sample ³	
1 to 50	2%	95%	Small Population Size or Highly Valuable Animals by Owner please contact APHIS for further instruction.	
51 to 100	2%	95%		
101 to 250	2%	95%		
251 to 500	2%	95%		
501 to 1000	2%	95%	163	
1001 to 2500	2%	95%	170	
2501 to 10000	2%	95%	175	
10001 to 40000	2%	95%	175	

¹ This number represents the sum of the total number of animals in all holding units in a population.

² If the animals to be sampled are highly valuable and cannot be sacrificed, contact APHIS to determine sampling levels.

³ Number has been rounded and calculated based on 85% test sensitivity and 100% test specificity. Any population above 40,000 is considered large (infinite).

3. Selecting specimens for the sample

- 3.1** Ensure that sample collection is conducted at the water temperature, time, season or under environmental condition that are conducive to detecting these pathogens (or that would facilitate the detection of the pathogen), if present. If sample collection cannot be taken at optimal environmental conditions, additional samples must be taken.
- 3.2** Capture animals in the most efficient and practical way possible. Try and ensure the samples are representative of the population distribution in the holding unit.
- For a single population in one holding unit specimens can be collected using a convenient method that allows for a representative sample from the population. When possible random selection of samples is preferred. Random selection means to choose samples such that each animal has an equal probability of being selected. It is recognized that true random sampling is difficult so sample selection may in fact be haphazard.
 - For sampling from a population contained in multiple holding units, the number of specimens to be collected from each unit should be **proportional** (see **Example** below) to the number of animals in each holding unit.
 - If sampling multiple populations in many holding units, sample each population described in (b).
- 3.3** Collect from a number of different locations in the holding units.
- 3.4** Collect and euthanize (MS-222 or Chlorine) animals in a humane manner.
- 3.5** Package and ship specimens per instructions of APHIS approved diagnostic laboratory.

Example

Sample Selection for the Collection of Animals from Populations Split in More than One Holding Unit:

Example #1. Sampling proportional to group size

Simple random sampling from a population comprised of several holding units (i.e., sub-population) is likely to produce sample sizes from each unit roughly in proportion to the number of fish in each unit. However, proportional stratified sampling will guarantee that each unit is represented in proportion. This simply involves dividing the sample size between units in proportion to their population.

It is assumed, based on available data, that the larger the group, the greater the probability of infection for the disease(s) of concern.

For example, as per Table 1, for a threshold prevalence of 2% and >40,000-population size, 175 animals need to be sampled if a premises' facility is comprised of:

Unit ID	Species	# of fish
1	Anguilla anguilla	25,000
2	Anguilla anguilla	60,000
3	Anguilla anguilla	135,000
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TOTAL		222,000

The first unit has 25,000 eels out of a total of 222,000. This represents 11% of the total population. Therefore 11% of the 175 animals that are to be sampled is 19. Therefore 19 should be taken from Unit ID #1. Using a similar approach, the number of fish to be taken from Unit #2 will be 47 (Unit 2 represents 27% of the total population) and for Unit #3 it will be 109 eels (Unit 3 represents 62% of the total population).