



TITLE

Collection of fleas from prairie dog burrows

1. PURPOSE

To describe step-wise procedures for the collection of live fleas from prairie dog burrows.

2. SCOPE

This SOP will cover all basic procedures for collecting live fleas from prairie dog burrows. In most cases, these fleas are suspected of carrying *Yersinia pestis*, the causative agent of plague. This SOP will be reviewed and updated any time procedures are changed.

3. RESPONSIBILITIES

It is the responsibility of all PMI personnel to follow this procedure. It is the responsibility of the PMI management team to review, revise and distribute SOPs for appropriateness and accuracy.

4. REFERENCES

None

5. REAGENTS AND MATERIALS

Materials

5.1 Pre-collection:

1. Prescription for appropriate antibiotic (e.g., doxycycline)
2. Light colored long sleeve shirts and pants
3. Light colored, fully closed shoes

5.2 For collection of live fleas:

1. 6 x 6 inch white or off white cloth (e.g., flannel) swatches
2. Drain snake with attached alligator clip
3. Disposable, extended cuff single-use nitrile gloves of the appropriate size
4. 3¾" W x 7" L Whirl-Pak® bags
5. Gallon Ziploc® bags
6. Large, durable plastic container with lid
7. Hand sanitizer or other disinfectant

5.3 Optional materials:

1. Insecticide/acaricide spray (e.g., OFF!®)
2. Disposable, single-use Tyvek® coveralls
3. Clear packing tape
4. Sunscreen

6. EQUIPMENT

1. Digital thermometer
2. GPS or equivalent application

7. SAFETY PRECAUTIONS

1. Appropriate PPE must be worn
2. When in the field, remain vigilant of other wildlife that may inhabit prairie dog burrows (e.g., rattlesnakes, badgers, spiders, etc.)
3. Exposure to sun can cause sunburn and dehydration; plentiful water and sunscreen are highly recommended
4. All personnel must monitor their temperature daily for 48 hours following flea collection (see Appendix I)
5. Be aware of the risks of DeltaDust, an insecticide used by Coconino County Health Department around the entrance to prairie dog burrows when a plague outbreak is suspected (<http://www.unexco.com/files/DeltaDustMSDS.pdf>)

8. DEFINITIONS

PPE – Personal protective equipment

9. PROCEDURE

Note: Occasionally, flea sampling will occur under high-risk conditions (e.g., in tall or damp grass, when fleas from a specific site have recently tested positive for high loads of *Y. pestis*, or when the percentage of positive fleas tested is >15%). It is recommended that under these circumstances additional precautions be taken to ensure the safety of project personnel. Section **9.4 Optional procedures** highlights additional steps that may be taken under these conditions, or anytime, at the discretion of the individuals working in the field.

9.1 Prior to flea collection

1. All personnel are required to meet with a physician at NAU Campus Health Services prior to beginning field work. The goals of these meetings are to:
 - a. Inform PMI staff and students about the occupational risks associated with flea sampling and exposure to *Y. pestis*.

- b. Provide education about strategies to reduce these risks.
 - c. Discuss the symptoms of plague infection with personnel who will be working in the field so that they may implement post-sampling surveillance.
 - d. Inform Campus Health Services so that they may be better prepared to diagnose and treat individuals in the event of a work related exposure.
 - e. Obtain a prescription for an antibiotic appropriate for treating *Y. pestis* infection.
2. Personal vehicles or vehicles from NAU Fleet Services can be used for driving to and from sampling sites. If an NAU vehicle is being rented, the driver must meet the following requirements of NAU's Authorized Driver Program:
 - a. Pass NAU's Defensive Driving course and quiz
 - b. Register their driver's license (including a review of their driving records)
 - c. Additional information can be found at <http://nau.edu/facility-services/safety-training/>
 3. If sampling on private land, permission must first be obtained either verbally or in writing from the owner of the land.

Note: Under most circumstances, Coconino County Health Department will have already gained permission from land owners to collect fleas from their property. Otherwise, it is the responsibility of PMI personnel to contact these individuals *prior* to sample collection. Sampling done near public right-of-ways or on Forest Service/BLM land does not require permission.
 4. All personnel must wear appropriate PPE. This includes:
 - a. A light colored, long sleeved shirt and light colored pants
 - b. Light colored, fully closed shoes with tight-fitting socks
 - c. Disposable, extended cuff single-use nitrile gloves of the appropriate size
 5. Long hair should be fully pulled back, so as not to be touching any clothing or the environment.

9.2 Collection of live fleas

1. To begin sampling, breathe onto a cloth swatch (2-3 deep breaths) to inoculate it with carbon dioxide.
 - a. Optional: Place the bated cloth at the outside of the burrow entrance to capture any fleas that could be at the surface.
2. Quickly fasten a bated cloth to a drain snake using the attached alligator clip.
3. Feed the drain snake into the burrow as far as possible
4. Wait ~10 seconds, then slowly retract the drain snake.
5. Carefully inspect the cloth for fleas. If fleas are observed, quickly fold them into the cloth and place the cloth in a nearby Whirl-Pak® bag. Roll the top of the bag down 3-5 times and secure with wire fastening strips while trying to minimize the amount of trapped air.
6. Repeat until no more fleas are retrieved

Note: Fleas collected from the same burrow may be kept in a single Whirl-Pak® bag, so long as the bag can be safely opened to add additional fleas.

7. Label the Whirl-Pak® bag with the burrow number, date, location, and approximate number of fleas. GPS coordinates may also be taken for each burrow.
8. Whirl-Pak® bags should be pooled in gallon Ziploc® bags and then placed in a large, durable plastic container with a lid prior to transport back to NAU.
9. Following flea collection, and before entering the vehicle, each member of the team should check themselves and be visually scanned by another individual for fleas. For this reason, a minimum of two people should be present at each sampling site.
 - a. When sampling in a high-risk area, these checks should be performed more frequently, at the discretion of the sampling team.

9.3 Following flea collection

1. All project personnel are required to monitor their temperature daily for a *minimum* of 48 hours following sampling (see Appendix I). If a fever (temperature $\geq 100.4^{\circ}\text{C}$), flu-like or respiratory symptoms develop, the individual should begin taking their prescribed antibiotics. If desired, they should also seek prompt medical attention and inform the attending physician of the possibility of infection with *Y. pestis*.

Note: Before sampling, each individual is given a prescription for an appropriate antibiotic. If a fever or other symptoms are detected when medical services are not available, it is recommended that the individual fill the prescription and begin taking their antibiotics as prescribed

9.4 Optional procedures

1. OFF!® bug repellent can be used following a collection event and should be made available for those who wish to use it.
2. Showers are available to PMI employees on the first floor of the Applied Research and Development (ARD) building #56, and can be used as a precautionary measure following flea collection.
3. Personnel may choose to doff and bag clothing worn for sampling and place it in one of PMI's BSL2 -80°C freezers overnight before bringing it home; exposure to -80°C temperatures is lethal to fleas.
4. Disposable, single-use Tyvek® coveralls can be worn over street clothes as an additional physical barrier when sampling in one of the aforementioned high-risk situations.
5. Clear packing tape can be used to create a tight seal between disposable gloves and long sleeve shirts, or around the ankles.
6. Additional flannel swatches may be inoculated with carbon dioxide and placed around the entrance to the burrow being sampled. When many fleas are present, this may reduce the chance of fleas jumping directly onto sampling personnel.

10. Additional comments

1. None

