APPENDIX I
SYLVATIC PLAGUE MONITORING PROTOCOL (DRAFT)
BLACK-TAILED PRAIRIE DOG CONSERVATION TEAM - MARCH 2002

Black-tailed Prairie Dog Conservation Team
March 2002 Draft Sylvatic Plague Monitoring Protocol

Dowd Stukel, E. South Dakota Game Fish and Parks Department
Bob Luce, Interstate Coordinator, Black-tailed Prairie Dog Conservation Team

BACKGROUND: Since its documented appearance in wild rodents on the Pacific Coast of North America in the early 1900s, sylvatic plague has spread eastward, affecting sciurid and cricetid rodents, insectivores, lagomorphs, carnivores, and humans (bubonic plague) (Barnes 1982, Cully 1993). Prairie dog species are extremely susceptible to this typically flea-borne disease and may serve as "amplifying hosts" (Barnes 1993).

Plague epizootics may originate from focal areas, with possible maintenance in non-focal areas between epizootics. During epizootics, plague can spread over great distances and in the process affect humans, most often during and shortly following epizootics (Cully 1993). Several wildlife species are considered enzootic or maintenance species for sylvatic plague, meaning individuals have some or considerable resistance to the disease. Examples include the California vole (*Microtus californicus*) in San Mateo County California, kangaroo rats (*Dipodomys* spp.), deer mice (*Peromyscus maniculatus*), and northern grasshopper mice (*Onychomys leucogaster*) (Cully 1993).

As part of a range-wide commitment to black-tailed prairie dog management, the Interstate Black-tailed Prairie Dog Conservation Team is developing specific strategies to monitor occupied habitat and threats to prairie dogs, including sylvatic plague (Van Pelt 1999). This document contains a framework for the design of a disease monitoring protocol for the black-tailed prairie dog.

PROPHYLACTIC TREATMENT: A technique used prior to prairie dog relocation in plague-affected towns is application of Deltadust Insecticide, which is labeled for control of fleas and ticks in rodent burrows (Dave Seery, pers. comm.).

PLAGUE SURVEILLANCE TECHNIQUES: Interest in monitoring sylvatic plague originated for two main purposes; protection of human health and protection of prairie dog populations for ecosystem values, in particular protection of reintroduced populations of black-footed ferrets. Potential sylvatic plague surveillance methods are summarized below.

Technique	Comments
"Windshield surveys"	General observations of prairie dog towns can be useful in detecting plague die-offs, with follow-up evaluations needed to confirm. Coordination with health professionals, field personnel, and private landowners important. Refer to CDC protocol.
Collection and analysis of dead prairie dogs	Prairie dogs often die in burrows. High mortality rate makes collection of live animals difficult. Refer to handling and shipping protocols.
Collection and analysis of fleas from prairie dog burrows	CDC recommendation; widespread applicability of this surveillance technique for human health concerns, included in the Shirley Basin/Medicine Bow black-footed ferret plague contingency plan (Luce and Oakleaf 1994). Young et al. (abstract only) reported on usefulness of this technique on Fort Belknap Agency, Montana, and the Pueblo Chemical Depot in central Colorado. Refer to CDC protocol (Enscore, pers. comm.)
Collection of blood samples from members of Order Carnivora likely to inhabit prairie dog towns	Although such species as badgers and coyotes can become infected with plague, their primary role in the disease cycle is the transport of plague-infected fleas (Poland and Barnes 1979 cited in Gage et al. 1994). Nobuto blood-sampling papers have been used extensively, since the technique does not require access to refrigerators and requires only 0.2 ml of blood (Wolff and Hudson 1974, Gage et al. 1994). Recently used extensively in association with black-footed ferret reintroduction, either via collection of blood samples from live animals or use of animals sacrificed for this purpose or killed during animal damage control activities (Anderson et al. no date, Williams et al. 1998, Matchett 2001). In addition, black-footed ferrets captured for removal of radio collars, for implantation of transponder chips, or for canine distemper vaccination can be bled for disease analysis samples. Technique can easily be incorporated into blood collection for other purposes, such as genetic analyses (NPWRC 1999).
Collection of blood samples from domestic dogs	Barnes (1982) reported on use of domestic dogs as sentinels for exhibiting antibodies to plague with little risk of death. Effective on Native American reservations in the Southwest in detecting seroconversion before plague was observed in rodents or humans.
Collection of blood from potentially resistant small mammals	Certain rodent species appear to be resistant to plague and may serve as maintenance or enzootic hosts that maintain plague between epizootics (Cully 1993, Gage et al. 1994). The Wyoming Game and Fish Department has monitored small mammals for plague seroconversion in Shirley Basin, Wyoming (Luce et al. 1996, Luce et al. 1997). Trapping efforts focused on deer mice and grasshopper mice, with the assumption that active plague would be detectable by antibodies produced during the short life spans of these rodents. These investigations detected a relationship between seroprevalence of plague in deer and grasshopper mice and status of prairie dog populations in Shirley Basin.

ACTIONS:

1. State wildlife agencies will initiate a public information program to inform landowners, hunters, and other members of the public concerning the need to notify the agency of die-offs of prairie dogs or ground squirrels.

2. State wildlife agency prairie dog coordinators, in cooperation with state public health officials, will take the lead to inform Department of Agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, or have regular contact with landowners and the public, of the need for reporting die-offs.

3. State wildlife agency prairie dog coordinators, in cooperation with state public health officials, will take the lead in providing information and training for Department of Agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, on protocols for collection of dead prairie dogs and ground squirrels, packaging, record keeping.

The CDC and Wyoming State Veterinary Laboratory (WSVL) both have extensive experience conducting disease surveillance in wild mammals. CDC does not charge for diagnostic services, but has limited laboratory capacity. The 11 black-tailed prairie dog states will use CDC, individual state diagnostic labs, or WSVL diagnostic services for examination of prairie dog and ground squirrel carcasses for disease detection. Although other laboratories can provide a similar service as the WSVL, there is significant advantage in having all of the diagnostic examination done at a lab that is familiar with the procedures, will produce consistent results, and will report them state by state for the 11-states as the WSVL has done for black-footed ferret reintroduction sites for several years. In addition to testing for plague, specimens will be tested for tularemia, pasteurellosis, undetected poisoning, drowning, and predator kill.

4. State prairie dog coordinators will coordinate development of windshield survey routes to be conducted annually by wildlife agency or other personnel in each county, or smaller unit, where prairie dogs occur, during March and April. Windshield surveys will follow the Centers for Disease Control and Prevention (CDC) protocol (Enscore pers. comm.)(Appendix 1). Significant decline in any colony or complex should be immediately reported to the state prairie dog coordinator.

5. Each state will have a contingency plan to put into effect immediately if a windshield survey route reports a potential die-off of prairie dogs or ground squirrels)(Appendix 2).

A. Make inquiries to determine whether or not the colony was poisoned, and whether mortalities were due to heavy shooting.

B. If neither shooting nor poisoning occurred, the colony or complex should be searched for prairie dog and ground squirrel carcasses as soon as possible after discovery of the population decline. Carcasses should be handled in the field according to protocol (Appendix 2).

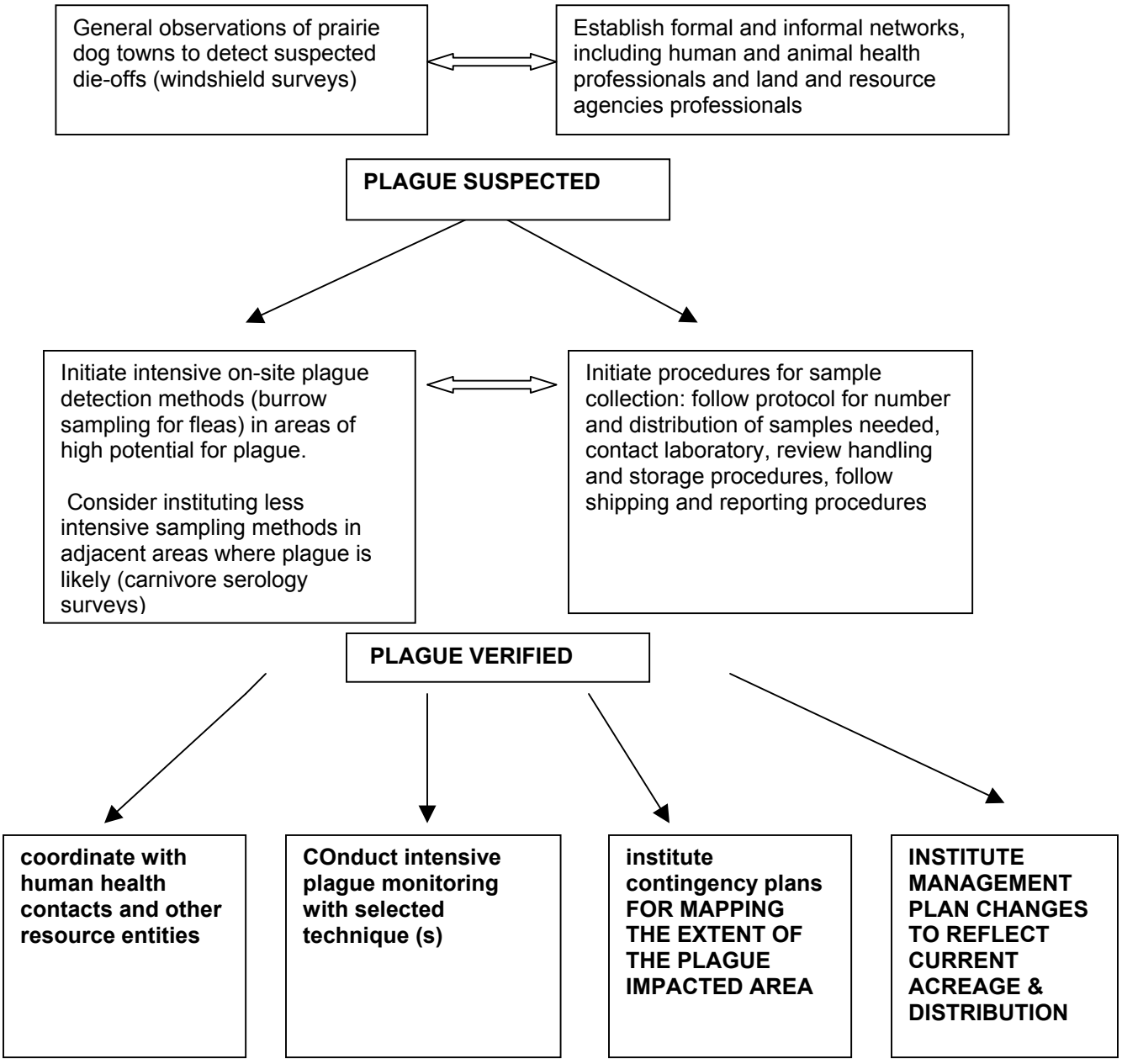
C. In the event that carcasses cannot be found, and the disappearance of prairie dogs is verified as recent, burrow swabbing should be conducted to collect fleas according to CDC protocol (Appendix 3).

6. If plague is verified, the prairie dog coordinator, in cooperation with state public health officials and CDC, should immediately notify the following: landowners and wildlife agency personnel in the affected area, department of agriculture, USDA-Wildlife Services, NRCS, veterinarians, and local government personnel that deal with animal control, the general public through local media sources.

7. Post-plague monitoring of prairie dog colonies should be conducted annually in March or April to document the rate of re-colonization and verify occupied acreage. Initial monitoring, which will take place from one to several years, should consist of windshield surveys. When visual surveys indicate prairie dog colonies are recovering, a quantitative survey method should be initiated. The recommended method, due to widespread use, particularly on black-footed ferret reintroduction sites, is transecting using the Biggins method (Biggins et. al. 1993), which equates active and inactive burrow densities to population density.

8. The prairie dog coordinator and the prairie dog working group should evaluate the extent of impact of the epizootic as it effects the acreage and distribution objectives in the management plan. The group should determine whether or not there is a need to modify prairie dog management in the plague area, and potentially elsewhere in the state, if occupied acreage is below the objectives in the management plan.

General Plague Monitoring Framework



- Consider managing the plague outbreak using chemical methods if the circumstances warrant (on a site by site basis)
- Consider translocation when post plague data collection indicate that recovery has begun (on a site by site basis)

Appendix 1

Centers for Disease Control
Procedure for Visual Evaluation of Prairie Dog Colonies for Plague in the Southwestern United States

Citation: Enscore, R. personal communication. Undated. Centers for Disease Control and Prevention, NCID, Division of Vector Borne Infectious Diseases, Plague Section, Fort Collins, Colorado. 3pp.

A. HEALTHY COLONY

OBSERVATION: The vast majority of burrows show signs of recent use, unless it has rained within the past 24 hours – in which case the colony should be reexamined following a period of at least 24 hours without precipitation. Active prairie dogs are observed during periods of acceptable weather conditions. Only a relatively few (<10%) burrow openings appear inactive (lack of disturbed dirt, presence of cobwebs or wind-blown vegetation over the entrance). An occasional carcass or dried bones may be present as a result of non-plague death or predation.

EVALUATION: Unless recently (days) introduced, plague is not likely to be present. Fleas are not likely to test positive.

SAMPLE RECOMMENDATIONS: No samples recommended.

B. DEAD COLONY

OBSERVATION: The colony appears completely inactive. Burrows show no signs of recent use (re-examine if it has rained within 24 hours). An occasional desiccated carcass and bones may be present, and have likely been scavenged.

EVALUATION: 1) Make inquiries to determine if the colony was poisoned. This is especially likely if it appears that dirt was shoveled into the burrows. If there is no evidence of poisoning and the food supply appears ample: 2) it is likely that plague or some other zoonotic disease killed the colony. An experienced observer can usually make an estimate (recently, 1 season, or 2 seasons) on how long the colony has been inactive by considering the soil type and degree of burrow degeneration.

SAMPLE RECOMMENDATIONS: Sample only if there is no evidence of poisoning. A recent (same season) die-off might produce many fleas through burrow swabbing. Older die-offs will likely produce few or no fleas. Typically, many burrows (dozens or even hundreds) may be swabbed with only a few producing fleas. If burrowing owls are using the inactive burrows, small black stick-tight fleas may be present in large numbers (in contrast to the larger, reddish-brown prairie dog fleas). Fresh or desiccated prairie dog carcasses may also be collected for analysis.

C. SCATTER PATTERN:

OBSERVATION: Inactive burrows constitute an unusually high (typically 20-90%) percentage of the total burrows. Active burrows however are clearly evident and active prairie dogs are observed during periods of acceptable weather. Active and inactive burrows are scattered amongst each other in no particular pattern (see below), keeping in mind that family units may have multiple burrow openings and hence an inactive unit may produce a small cluster of 2-5

inactive burrow openings. An occasional carcass (fresh or desiccated) and bones may be present.

EVALUATION: Several scenarios could account for these observations – and more than one scenario may be in play at the same place and time. Presented in order of likelihood: 1) Make inquiries to determine if the colony was poisoned. This is especially likely if it appears that dirt was shoveled into the burrows. This scatter pattern could be produced if the application of poison was scattered and not comprehensive, 2) If there is no evidence of poisoning, assess the available food supply. Such a pattern of death could also be attributable to a population crash as a result of lost carrying capacity of the site or over-population, 3) If there is no evidence of poisoning or population crash, hunting by humans or excessive predation by carnivores or birds of prey are highly likely. Human hunting usually produces physical evidence such as footprints, tire tracks and spent ammunition shells. Depending upon the local culture, human hunters may collect their prey (many Native American groups regard prairie dogs as a delicacy) or leave it for scavengers. Experienced observers can often spot carnivore tracks and recognize hunting and attack patterns in these tracks near burrow entrances, 4) Finally, a zoonotic disease could be responsible, but given this mortality pattern, a disease with a lower mortality rate than plague is more likely.

SAMPLE RECOMMENDATIONS: If there is no evidence of poisoning, population crash, or excessive human hunting: collect fleas by swabbing burrows – especially inactive burrows – and collect fresh or desiccated prairie dog carcasses if available.

D. DEAD ZONE

OBSERVATION: Within an otherwise healthy appearing colony, there is a zone of inactive burrows. This zone may encompass a relatively small or large proportion of the colony, and may be located anywhere in the colony. Eventually it spreads to encompass a section of the colony and appears to be spreading, along a discernable line of demarcation over the remaining section of the colony. Experienced observers can often clearly distinguish and mark (flagging tape) this demarcation line between active and inactive regions. Marking allows for periodic re-examination to assess the rate of spread and facilitates sampling. Fresh or desiccated carcasses may be present. Near the demarcation line, recently inactive burrows may reveal the odor of decaying carcasses and flies may be common at burrow entrances.

EVALUATION: 1) There is a high probability that plague is active in such a colony. Although other zoonotic diseases are possible, plague is most likely, 2) Depending upon the location of the dead zone with respect to other human activity (homes, barns, etc.) poisoning is also a possibility and should be investigated.

SAMPLE RECOMMENDATIONS: Collect fleas by swabbing burrows immediately along both sides of the demarcation line, concentrating a majority of your efforts immediately along (within 10meters) the inactive (dead) side of the line. Fleas are likely to be numerous. You may wish to apply extra insect repellent but be extremely cautious not to directly or indirectly get repellent on your burrow swab! (If this happens: discard it, wash your hands, and start with a new one). If others in a group are getting fleas and you are not, and you are swabbing essentially the same area, you likely have repellent on your swab. Collect any available rodent carcasses (fresh or desiccated, prairie dog or other rodent) for testing.

Additional Notes: Please include GPS coordinates for all samples. One set of coordinates per colony is acceptable. Specify the type of inactivity pattern noted for each sampled colony: dead colony, scatter pattern, dead zone. Analysis of samples from “dead zone colonies” will receive laboratory priority.

The above activity patterns are typical for the warm months. Visual examination during winter months is more difficult due to decreased daily activity among even healthy animals.

Appendix 2

Field Procedures for Collecting and Handling Carcasses as Diagnostic Specimens

1. Search prairie dog colonies systematically using walking or 4-wheeeler transects spaced at about 50 meters.

2. When a carcass is discovered, ascertain if possible, whether or not the animal was shot. If mortality by shooting is confirmed there is no need to collect the specimen.

3. Before you collect a carcass, prepare a tag with the following information: species, date, location (both legal description and UTM is recommended), name of collector, agency or affiliation of collector, telephone number and address of collector, brief description of circumstances for collection.

4. When collecting a carcass, the collector should wear leather or latex gloves, and a long sleeved shirt or jacket that is tight at the wrist, to ward off fleas.

5. Invert a one-gallon plastic ziplock freezer bag over your hand, grasp the carcass in your hand, quickly fold the bag over the carcass, roll the bag on the ground, away from your body, to expel the air, and seal the ziplock.

6. Immediately place in a second ziplock bag, put in the tag, roll and seal the second bag.

7. As soon as possible after collection, freeze the specimen.

Sample Size:

1) If specimens are from a single sample area (one prairie dog colony or area) collect as many specimens as is practical up to 15, but initially ship only the freshest five specimens to the diagnostic lab.

2) Freeze the additional specimens that were collected, up to ten, and save for further testing needs, depending upon the results from the testing of the first five specimens. Keep the samples until notified by the WSVL or other lab that results were obtained form the first five samples and that the additional specimens will not be needed.

Ship the frozen specimen to WSVL, CDC, or designated lab.

(DO NOT USE UPS). U.S. Postal System or FEDEX can ship carcasses that are sealed in plastic bags and a cardboard box. Their regulations require:

1) Carcasses must be individually labeled and bagged in watertight bags (minimum triple bag in ziplocks)
2) Placement of absorbent packing material around the carcass (crumpled newspaper, etc.

3) Use of approved laboratory shippers or hard-sided containers, adequately taped closed

4) Marking of the container with “Biomedical Material” label (for U.S. Postal Service) or shipped as hazardous material by Federal Express (requires a special form and should be labeled as Diagnostic Biomedical Material on the form. Labels and forms may be obtained from the U.S. Postal Service or Federal express.

5) Carcasses should be frozen or packed with frozen ice packs (no wet ice).

Cost: WSVL cost for testing for plague, tularemia, pasteurellosis, undetected poisoning, and predator kill is a maximum of $60.00 per specimen. CDC testing is free but the Ft Collins laboratory has limited capacity and can handle no more than 50 specimens per year.

Contact before shipping:

Dr. Beth Williams
Wyoming State Veterinary Lab
1174 Snowy Range Road
Laramie, WY 82070
307-742-6638

or

(Shipment by U.S. Postal System)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
P.O.Box 2087
Ft. Collins, CO 80522

(Shipment by FEDEX)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
Rampart Road (CSU Foothills Campus)
Fort Collins, CO 80521

Appendix 3

Centers for Disease Control
Procedure for Flagging (Swabbing) Rodent Burrows

Citation: Gage, K. Personnel Communication. Undated. Centers for Disease Control, Ft. Collins, CO. 3pp.

Leon Carter: 970-221-6444 (Biologist, Diagnostic and Reference Section - Responsible for handling specimens and doing much of the plague-associated laboratory work at CDC.)
Ken Gage: 970-221-6450 (Plague Section Chief - Responsible for CDC's plague surveillance And control program. Trained as medical entomologist/zoologist)
Rusty Enscore: 970-221-6452 (Environmental Health Specialist IV, Plague Section - Registered Sanitarian)
John Montenieri: 970-221-6457 (Biological Technician, Plague Section - GIS specialist)

Some important flea vectors of plague infest rodent species that live in burrows. Although these fleas usually can be found in abundance on live hosts, they also can be collected by a procedure known as burrow flagging or burrow swabbing.

This procedure requires:

1) **Burrow swabbing device** consisting of a flexible cable, wire, or strong rubber hose with spring-loaded clip attached to the end. We prefer a steel plumber's "snake" that has an alligator clip screwed on the end as a means of attaching the flag. A simple burrow swab can be made by attaching a flag to the end of a piece of wire (about the thickness of a coat hanger), but this primitive swab allows only the top 2 or 3 feet of a burrow to be swabbed and will miss some fleas. Despite the shortcomings of the latter technique, it can be useful when die-offs are encountered unexpectedly and more sophisticated means of swabbing fleas are not available.

2) **Flags** consisting of white flannel cloth squares (approx. 25 cm^2 or 10 in^2). We prefer white flannel because it is easier to see the fleas on white cloth than on cloths of other colors. Flannel is better than most other cloths because of its deep nap, which increases the likelihood that fleas will continue to cling to the cloth flag after it is removed from the burrow.

3) **Plastic bags** (approx. 20-40 cm^2 or 8-15 inches)(Zip-loc type are best)

4) **Insect repellent** (DEET) to spray on clothes and exposed skin on arms, legs, etc. Although this is recommended for safety reasons, care must be taken not to apply repellents to hands because the repellent is likely to transfer to the flagging material, thus preventing fleas from jumping onto the flag. Note: Clothing also can be treated with permethrin-containing sprays but these sprays should not be applied directly to the skin.

Procedure:
1. Attach a flag to the clip on the end of the burrow swab.

2. Force the flag as far as possible down the burrow. The fleas confuse the flag with their normal host and cling to it as it passes through the burrow.

3. Slowly withdraw the flag from the burrow after approximately 30 seconds.

4. Quickly place the flag in a plastic bag.

5. Seal the bag to prevent the fleas from escaping.

6. Keep track of the number of burrows swabbed so that a burrow index can be calculated.
   Burrow index = no. fleas collected/no. burrows sampled - This value often increases dramatically during die-offs among prairie dogs, rock squirrels, California ground squirrels, or other ground squirrel species)

7. Place another flag on the swab and repeat steps 1-6 for each burrow.

8. Transport flags back to laboratory in the plastic bags. Keep the bags in a reasonably cool place to prevent dessication of the flea samples (*Yersinia pestis* is very susceptible to death by dessication) or death of the plague bacilli due to excessive heat (remember pick-up hoods can get very hot in direct sunlight! Fried samples will come back negative for plague everytime!).

9. Place bags in freezer overnight to kill the fleas.

10. Place the flags and loose contents of the plastic bags in a white enamel pan. Fleas may be picked from the flags and bottom of the pan with forceps.

11. Place fleas in vials containing 2% saline and a very small amount of Tween-80 detergent (<0.0001% of solution). Remember the detergent is added to reduce surface tension and allow the fleas to sink to the bottom of the vial. Too much detergent will kill the plague bacteria and prevent successful isolation. Fleas can be submitted in 2% saline without Tween-80, but an effort should be made to submerge the fleas. If the fleas have been killed by freezing, this should not be a problem. Although not recommended for routine collecting, some investigators occasionally remove live fleas directly from the flags and place them in vials of saline. Live fleas placed in saline containing the Tween-80 detergent will be unable to float on the surface of the liquid, thus ensuring that they will drown soon after being placed in the saline. Without the detergent, surface tension can become a problem because the numerous bristles and setae found on fleas enable them to remain afloat on the surface of saline. This can be a potential safety problem because floating fleas often survive shipment and arrive at the laboratory ready to jump from onto lab personnel. Rapid freezing of the fleas obviously eliminates this problem, but adding Tween-80 to the saline also helps reduce the growth of fungi on flea samples. Dead fleas trapped in the surface tension at the air-saline interface rapidly become overgrown with fungi making identifications more difficult.

12. Vials containing 2% saline and fleas can be shipped to CDC for taxonomic identification and analysis of the fleas for *Yersinia pestis* infection. The fleas can be shipped at ambient temperature in the vials of 2% saline. For best results, ship the specimens as soon as possible because the fleas will start to decay soon after collection. Be sure and double wrap the vials in a leak-proof material and then place them in a crush-proof box or metal mailing tube for shipment to CDC.

13. CDC Address:

(Shipment by U.S. Postal System)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
P.O.Box 2087
Ft. Collins, CO 80522

(Shipment by FEDEX)
CDC/Bacterial Zoonoses Branch
c/o Mr. Leon Carter
Rampart Road (CSU Foothills Campus)
Fort Collins, CO 80521

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