

## Preparation and submission of Specimens for Laboratory Examination

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The capability of a laboratory to confirm the diagnosis of a suspected infectious animal disease is directly related to the types, amount and conditions of the specimens submitted. The field diagnostician must select, aseptically procure, and properly preserve specimens for the isolation or demonstration of a causative agent. In addition, an adequate number of specimens must be taken from the appropriate tissues, at the proper stage of the disease, to maximize the chances of isolating the pathogen. The herd or flock owner, private practitioner and diagnostic laboratory comprise a front-line defense and will, most likely, be confronted with the initial case of an infectious animal disease. It is vitally important that these people contact the state Veterinarian or Veterinarian in charge district diagnostic laboratory as soon as possible if contagious disease is suspected. The diagnostician is responsible for collecting and dispatching specimens for further investigations if needed, at laboratory of Veterinary college of respective state.

When the existence of an exotic disease is suspected, no animal or specimen should be removed from the premises of origin unless in the custody of an officially designated diagnostician.

### Preparation of Specimen for Disptach

An initial incursion of an exotic disease in most cases, only be confirmed in a reference laboratory through the isolation and identification of the etiologic agent. Thus specimens to be submitted for agent identification should be collected strict aseptically and completely.

The following general suggestions are presented as a guide for preparing diagnostic specimens for submission to a diagnostic laboratory.

1. Obtain and record a complete herd history. Information should be submitted on proper forms, when possible the following information should be included.

- Name and address of the owner.
  - Name, address and phone number of submitter.
  - A description of animal : Breed, Sex, Peculiarities etc.
  - Suspected disease or examination requested or both.
  - Number of animals showing similar symptoms and age of the animals.
  - Number of animals died due to the condition.
  - Vaccines administered to the animal(s) from which specimens were collected especially important when examinations for antibodies required.
  - Date of the first losses and subsequent losses.
  - The disease symptoms and their duration.
  - Ration fed.
  - The general condition of the animal.
  - A description of the spread of the infection, if in a flock or herd. A diagram of the area is often useful.
  - Treatment given if any.
  - Type of housing.
  - Accessory information like the type of preservative used for specimens.
  - An epidemiological assessment, including recent movements into and out of the flock or herd.
  - Any exposure of the affected poultry or livestock to persons.
2. Be objective and approach the investigation without a preconceived diagnosis.
3. Be alert regarding safety hazards in handling livestock and consider zoonotic potentials e.g.possibilities of rabies as a differential diagnosis should be considered where appropriate.
4. Ensure that prelabeled specimen containers and tubes are available for collection and are scrupulously clean and sterile. The label must

include proper identification of the animal and type of specimen.

5. Examination and collection of specimens from live animals or poultry in various stages of clinical disease. Serum, vesicular fluid or tissue, or both, swabs of exudates or lesions or both can be secured from live animals. Serum from apparently healthy exposed animals or poultry can also be helpful. Animals sampled should be permanently identified because it is possible that convalescent serum or sample will be taken in future for comparative purpose.

6. Blood smears should be prepared on clean glass slides. A thin blood film should be made, rapidly dried, and fixed in absolute methanol for 5 minutes. Slides having frosted end should be used and should be identified using a lead pencil.

#### Collection of Specimens at Necropsy

1. Collect specimens from the animal which have undergone minimal putrefaction.

2. Be aware of any safety or biological hazards that necropsy might impose on you and the owner. Availability of a proper and safe disposal site should be considered before beginning necropsy.

3. Wear rubber boots, gloves, overalls etc. that can be disinfected or that are disposable. A mask and goggles may be used at the discretion of the diagnostician.

4. Pre-labeled specimen containers will help ensure that recommended specimens will be collected.

- Use a label that can not be easily destroyed.
- Writing should be with pencil or ink that will not smudge or blur when wet.
- Use plastic screw-cap container instead of glass container where practical.
- Tape the lids of containers tightly so there will be no leakage as well as entry of other material.
- Use disposable equipment such as cardboard trays, disposable syringes etc.

5. Have a systematic plan for the necropsy and know what specimens are to be collected before starting the procedure. Be certain to include all lesions for laboratory examination. Body fluids and contents of cysts, abscess, or skin lesions can be collected using a sterile swab. If an animal is presented for euthanasia, collected blood sample before euthanasia. If the animal or bird is presented dead, collect blood from the heart. Make blood smears as per standard method. Ectoparasites should be noted

and collected, if present. The collection of specimens based on species rather than a specific disease will be most useful in providing a diagnosis in some diseases. Samples of all lesions should be collected for histological examination.

6. Fluid from any enlarged joints, if found should be aspirated aseptically.

7. Any excess body cavity fluids should be collected aseptically via a syringe.

Some other considerations in Specimen collection

1. Two sets of tissues are to be collected.
  - Fresh tissue for microbiological examination.
  - Preserved tissue for histological examination.

The recommended preservative is 10% neutral buffered formalin. All tissues can be placed in one container, but allow not more than 1 volume of tissue to 10 volumes of formalin. Tissues from organs should be cut perpendicularly to the surface to expose their anatomic structure. The specimen should include affected and surrounding normal tissue. To provide adequate fixation, tissue except brain, should be sliced no more than 3-6 mm thick. Any lymphnodes collected should be incised.

Specimens should not be folded or bent the container in which they are fixed. Only wide mouthed containers should be used in this procedure.

2. The initial piece of each organ or lesion should be collected aseptically for microbiology. Tissues for formalin fixation can be collected during the necropsy.

3. Swabs should be sent in appropriate transport medium. The laboratory can assist in the procedure for obtaining the media.

4. Materials submitted for possible virus isolation should be obtained from animals that died and have minimal putrefaction and from animals in the early, acute, febrile phase of illness. Specimens shipped for virology and bacteriology should be shipped refrigerated. If at all possible, the use of dry ice should be avoided because of CO<sub>2</sub> will produce acid conditions that will inactivate many viruses. If there is no way to submit the specimens to the laboratory within 48 hours, dry ice must be used. In this case, the specimens must be completely sealed so that there is no contact of the gas emitted by the dry ice with the specimens.

#### Post necropsy considerations

1. Clean and decontaminate all instruments and premises.
2. Record necropsy findings.
3. Dispose of carcass and body parts so as to

avoid exposure to other animals and contamination of environment.

### Considerations for shipping diagnostic specimens

Regulations require that diagnostic specimens transported in interstate or inter country traffic must be packaged and labeled properly. Improper packaging and labeling of diagnostic specimens and other hazardous material can result in unnecessary exposure to postal, shipping, laboratory personnel etc.

1. The specimens must be in securely closed, waterproof primary enclosure such as screw cap container or sealed vial. Be certain that exterior surfaces of the primary containers are decontaminated before shipment/air parcel.
2. Each primary container should be wrapped in sufficient dry absorbent cotton or paper towels to absorb the material in case of breakage. Ideally, the wrapped container should be placed in sealed plastic bags.
3. Pint, quart or half gallon sized paint cans should be used as secondary containers. These cans should have friction type lids and be water proof when hammered closed. The primary container should be padded with more cotton or paper to prevent jarring. A tertiary container, such as large sized version of the secondary container, should be considered if a zoonotic or highly infectious disease is suspected.
4. The sealed secondary or tertiary container should be placed in a shipping container and again packed with material such as paper. The shipping

container should be an insulated box with a lid that can be taped shut. A corrugated shipping box, affixed with the proper labels and shipper's certification, is the final enclosure and contains all other containers.

5. If specimens can be in transit for less than 48 hours, ice packs may be used for cold storage. Frozen "foam ice", "blue ice" picnic packs, or water frozen in sealed containers may be used. Wet ice, even when wrapped in plastic bags, should be avoided to eliminate the possibility of leakage.
6. Dry ice is the only suitable refrigerant to keep specimens frozen. Shippers must be aware of dry ice restrictions imposed by certain airlines and plan accordingly.
7. Regular mail or airmail shipment should not be used when an exotic infectious animal disease is suspected. Courier service is the appropriate method of shipment. If FMD is considered as a possible diagnosis, a responsible individual should hand carry the specimen to the reference laboratory.
8. If it is not desirable to have the submission form, with the history and other information, within the container. It is preferable to enclose the submission form between the shipping container and the cover of the outside corrugated box.
9. The shipper is responsible for notifying the intended recipient of all information related to transportation arrangements in order to expedite package pickup and delivery to the laboratory.
10. Care must be taken to ensure that an exotic infectious animal disease suspicious package is only opened within the confines of a biosecure facility.

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## Tuberculosis in Bovine-California, USA

More than 4800 dairy cows at risk of carrying tuberculosis (TB) are being slaughtered this week in central California, where nearly 16 000 cattle in the country's largest milk-producing region have been quarantined, federal officials said. Federal and state agriculture officials were still tightlipped about the identities and locations of the 3 dairies where cows tested positive for the disease, which can be transmitted to humans and other mammals through the air or through drinking unpasteurized milk from an infected cow. The discovery of the highly contagious respiratory disease already has prompted changes in interstate shipping regulations. Pasteurization kills the TB bacteria, but trade organizations still worry about the possible financial impact on California's USD 7.3 billion dairy industry.

One of the affected dairies milks more than 10 000 cows and sells semen and embryos from high-production cows and bulls internationally. The operation may face losing 50 years of genetic development if the dairy operator chooses to slaughter his cattle. The restrictions on transport make it more difficult for ranchers to sell cattle out of state, transport them for out-of-state grazing during California's dry season, and for breeders to haul livestock to shows.