

AGRICULTURAL EXPERIMENT STATION

KANSAS STATE AGRICULTURAL COLLEGE
MANHATTAN, KANSAS

DEPARTMENT OF VETERINARY MEDICINE

PREPARATION AND SHIPMENT OF SPECIMENS FOR LABORATORY DIAGNOSIS¹

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The purpose of this circular is to give information concerning the selection, preparation, packing, and shipping of animal tissues, organs, etc., to a laboratory for diagnosis. The methods described must be carefully followed if an accurate diagnosis is to be expected in a reasonably short time. If a specimen is received in an advanced stage of decomposition, its ultimate end is the incinerator and the report sent out is to this effect: "The tissue which you sent in for diagnosis was received in an advanced stage of decomposition. Contamination was present to such an extent that it was impossible to make an accurate diagnosis."

A rapid, accurate diagnosis is only possible under the most favorable circumstances. The degree of accuracy and the rapidity of diagnosis decrease in direct proportion to the degree of decomposition of the specimen. For instance, anthrax is suspected, and a specimen of spleen and liquid blood is placed in a Mason jar which is not packed in ice and sawdust, though the weather is hot. Upon arrival at the laboratory the specimen is found to be a decomposed mass, which has generated almost sufficient gas to break the jar. When the lid is unscrewed, the gas rushes out and blood spurts in every direction. The laboratory man is thus subjected to infection with anthrax, due directly to carelessness on the part of the shipper through improper selection and packing of the specimen. It is also important that this fact be kept constantly in mind; namely, that many pathogenic microorganisms are so delicate in growth requirements that they die when shipped under im-

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proper conditions, for the reason that the essential elements needed for their nourishment have been so changed by contaminating organisms that they can no longer obtain the necessary food for growth and reproduction. This is quite frequently the case with the *bacillus anthracis*. Under such circumstances, instead of receiving a positive diagnosis of anthrax, which should be the case, a report of post mortem decomposition is received.

METHODS OF DIAGNOSIS

There are two distinct methods of examination in laboratory diagnosis; namely, pathological and bacteriological. In many instances a pathological examination will suffice in making a diagnosis. In other instances only a bacteriological examination will result in making the proper diagnosis. Infectious diseases are caused, in most instances, by bacteria, and therefore the finding of the causative organism would result in a positive diagnosis; whereas, in the case of *tumor, nephritis, hepatitis*, etc., a bacteriological examination would be of small importance and a pathological examination would be the method. The method of packing tissues is distinct for each.

PREPARATION FOR BACTERIOLOGICAL EXAMINATION

In the bacteriological examination it is to be remembered that the tissues must reach the laboratory in a fresh or a good state of preservation, and must contain living organisms. Therefore antiseptics, preservatives, or anything having a destructive action on bacterial life must necessarily be kept away from the tissues. On the other hand, tissues for pathological examination may be placed in preservatives or fixatives, without harming them for examination, as living organisms would not be considered in this case.

PREPARATION FOR PATOLOGICAL EXAMINATION

In transmitting specimens for pathological examination it is sufficient to immerse them in a 4 percent solution of formalin. Of course, judgment should be exercised in obtaining a portion of tissue that appears to be severely affected. The tissue should be cut into pieces about one-half of an inch square, and two or three of these squares from each different organ or tissue to be examined should be placed in a water-tight glass container. The containers should be prepared in accordance

with "Postal Laws and Regulations," an extract of which is appended to this circular. If it is desired to send entire organs for diagnosis, they should be prepared in accordance with the appended extract of "Traffic Laws and Instructions" and sent to the laboratory by express.

SELECTION AND PREPARATION OF SPECIMENS FOR SHIPMENT

It is the intention to consider the most common diseases of animals and to point out the tissues to be selected from animals dead of each disease which from a laboratory viewpoint furnish the most desirable tissues for diagnosis; also to outline a proper, simple, and inexpensive method of preparing these specimens for shipment.¹

ANTHRAX

When anthrax is suspected, sufficient material for a laboratory diagnosis can be obtained by removal of one ear. Post mortem decomposition begins in the intestines, and then gradually spreads outward, backward, and forward, so that the extremities are the last to be affected. The ear, therefore, being classed as an extremity and having a small blood content, furnishes a desirable specimen for diagnosis. The ear is the best selection from the practitioner's standpoint, as it can be readily removed without any danger of contaminating one's self or the premises.

To remove the ear, first wash it with soap and water so as to eliminate contaminating influences as far as possible. It should then be ligated (tied off) close to the head and cut just below the point of ligation. Sear both cut surfaces of the ear by using a red-hot knife blade, branding iron, or a firing iron, and place it in a cleaned and boiled Mason jar (inner container). The jar should be sealed perfectly and placed in a tin (outer) container. Cracked ice and sawdust should then be placed in the outer container in such a manner as to completely surround the inner container. The containers should then be securely and carefully wrapped, addressed, and *immediately* shipped to the nearest laboratory for diagnosis.

BACTERIAL DYSENTERY OR PARA-TUBERCULOSIS

In this disease a section of the ileum about six inches in length should be shipped to the laboratory for diagnosis if the laboratory is close at hand. If the laboratory is quite a dis-

¹ For convenience, the diseases and types of specimens are listed in alphabetical order.

tance away, several small sections about one-half of an inch square should be cut from a portion of the intestines where the folds are highest. These should be placed in a small glass receptacle containing a 4 percent formalin solution. This should be placed in a tin container and be so arranged as to have at least one-half of an inch of compressed paper separating it from the tin container.

BLACKLEG

The choice selection in this disease is a piece of muscular tissue taken from the affected region. A piece two inches square is sufficient. It should be covered with salt and placed in a glass container. The glass container should then be placed inside of a tin container and surrounded by cracked ice and sawdust, carefully sealed, wrapped, and, *immediately* shipped to a laboratory for diagnosis.

BLOOD

When it is desired to send blood to the laboratory for serological diagnosis, the following outline should be carefully followed: The number of samples of blood to be taken should be estimated and a small bottle (1 ounce) prepared for each animal to be tested. The bottles and stoppers should be boiled in a solution of 3 percent carbolic acid for 15 minutes. The carbolic acid solution should then be drained from the bottles, and the bottles stoppered and labeled and wrapped in clean paper until they are to be used. The blood should be taken from the jugular vein after an area over it has been thoroughly cleaned and disinfected. The needle to be used should previously have been sterilized by boiling. The bottles should be filled almost to the neck and should then be placed in a container surrounded by cracked ice and sawdust, and packed so as to prevent breaking through rough handling in shipment.

If a bacteriological examination of the blood is desired, it should be collected and packed as above described. In addition, several blood smears should accompany the blood sample. The blood smears are made as follows: Glass slides are cleaned with alcohol and dried with a clean cloth. A drop of blood is placed in the center of one end of the slide, and the end of a second clean slide is placed on the drop of blood. By capillarity the blood follows the edge of slide number two; it is spread by pushing or pulling the second slide over the first, holding the

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second slide at an angle of about 45° and using moderate pressure. The smear should cover the entire slide and should be just thin enough to be noticeable on the slide. The blood films should then be allowed to air dry before they are wrapped. To securely and safely wrap the slides for shipment, several thicknesses of paper should be interposed between them.

EXTERNAL PARASITES

Ticks (*Margaropus annulatus*), lice, or any other external parasites should be forwarded to the laboratory for identification in a tightly stoppered, small glass vial. Several specimens should be sent and the bottle should be wrapped in accordance with the appended "Postal Laws and Regulations."

Material from mange suspects should be obtained by deeply scraping the affected parts with a sharp scalpel. The scraping should be continued until blood is made to ooze out from the scraped area. Scrapings must not be taken for at least 24 hours after the application of treatment, as the treatment is liable to kill all the parasites in the superficial layers of the skin, and may render their detection difficult or impossible. The scrapings should be placed in a small glass vial, tightly stoppered, and the vial should be securely packed in a mailing case so as to prevent breakage in shipment through the mail.

FECES

Fecal matter should be collected in a clean, tightly stoppered bottle. Surround the bottle with cracked ice and sawdust, to retard decomposition, in a water-tight tin container.

GLANDERS

Nasal discharge should be carefully taken from the nose on a sterile cotton swab, and the swab then returned to its sterile container. The container or test tube should be plugged with sterile cotton, sufficiently tight to keep the swab from coming out. The cotton plug should be pushed about one-half of an inch below the mouth of the test tube and covered with melted parowax so as to prevent evaporation of moisture. This then should be placed in a mailing case and should have sufficient cotton surrounding it to prevent breakage in transit. Along with the nasal discharge, send a sample of blood obtained in the same manner as before described. If the animal is dead or has been destroyed, excise (cut out) portions of skin, nasal mucous membranes, or lungs which show lesions. These tissues

should be placed in a 4 percent formalin solution in one container, or if convenient, a separate container for each different tissue can be used.

HEMORRHAGIC SEPTICEMIA

Portions of pneumonic lungs and portions of liver showing focal necrosis and infarcts should be excised (cut out) from a cadaver and sent to the laboratory as before described. In the living animal, blood samples should be taken as before described and sent to the laboratory. To avoid all fear of contamination, the large vessels of the heart can be securely ligated (tied off), so as to prevent egress of blood or ingress of surrounding fluids into the heart, thus contaminating the contained blood. The whole heart can then be removed from the cadaver, packed, and sent to the laboratory.

HOG CHOLERA, SWINE PLAGUE, AND SWINE ERYSIPELAS

Portions of the lungs, liver, intestines, kidneys, spleen, skin, and lymph glands should be packed as other specimens heretofore described, and shipped immediately to the laboratory. The intestines should include the ileocæcal valve. The entire heart should be prepared as described under hemorrhagic septicemia and sent along with the other specimens.

INFECTIOUS ABORTION

The entire fetus and membranes, if possible, should be wrapped in a clean cloth and placed in a tin container surrounded by cracked ice and sawdust, both containers to be water-tight.

Blood should also be drawn from the jugular vein of the dam, as described under blood, and submitted to the laboratory for diagnosis.

INTERNAL PARASITES

Tapeworms, roundworms, and flukes should be washed free of fecal material and placed in a 4 percent solution of formalin, a 60 percent alcohol solution, or a $1/1000$ bichloride of mercury solution.

MALIGNANT OEDEMA AND TETANUS

Portions of infected tissues should be excised (cut out), placed in a clean sterile bottle, packed in ice and sawdust as before described, and shipped to a laboratory with as little delay as possible. A piece of tissue about four inches square is sufficient.

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MILK

For bacteriological analysis the milk should be collected as described below in detail. The udder should be washed with warm water and soap and a weak antiseptic solution applied. This solution should be allowed to dry so that liquid material will not drop from the udder while the sample of milk is being taken. The hands of the operator should be thoroughly washed, and the container into which the milk is received should have been previously boiled. The first four or five streams are discarded and then the container is filled with milk. A half pint of milk will furnish sufficient material for examination, but a larger sample is better. The milk should be placed in a water-tight container and surrounded by cracked ice and sawdust and packed carefully to avoid breaking in shipment. A letter should always accompany such shipment stating the nature of the examination desired, as fat determination, bacteriological examinations, etc.

PUS

Pus should be collected on a sterile cotton swab. These may be obtained by writing to the laboratory for them. They are inoculated by holding the test tube in the left hand and removing the cotton plug and swabbing with the right hand. Rotate the cotton swab in the pus, after having cleaned away all the contaminating material, and replace the swab and cotton plug in the test tube. Trim the cotton plug even with the top of the test tube with scissors and seal the top over the cotton and around the wire perfectly with sealing wax or melted parowax. This prevents, to a certain extent, evaporation of moisture. The swab should then be brought in person to the laboratory. If this is impossible, it should be wrapped in accordance with "Postal Laws and Regulations" and posted immediately.

RABIES

In this disease, if possible, it is best to allow the animal to die a natural death. If it is not possible to get the animal "penned up," and the animal is shot, cut off the head, leaving as much of the neck with the head as is possible. This enables the laboratory man to locate the ganglia to be examined in conjunction with an examination of the brain. After removal of the head and neck close to the trunk, the animal should be care-

fully autopsied in order to exclude everything else, such as internal parasites, etc. A letter should accompany the head stating exactly what was found in the dog, or other animal, on post mortem. The head should be placed in a tin, water-tight container, be surrounded by cracked ice and sawdust in a second water-tight container, and be labeled and addressed in accordance with the "Traffic Laws and instructions" of express companies.

TUBERCULOSIS

Small specimens may be forwarded to the laboratory in a tightly stoppered bottle or tin, water-tight container, packed in accordance with the appended regulations. Large specimens should be wrapped in sterile or clean gauze, encased in a water-tight tin container and surrounded by cracked ice and sawdust in a second water-tight tin or other metal container.

TUMOR

If the tumor is small it should be removed entirely and sent to the laboratory. If large and a diagnosis is desired, before removal of the tumor a few specimens about one-half of an inch square should be excised and placed in a 4 percent formalin solution in a water-tight bottle. If the tumor is a large one and has been removed, it should be cut in quarters in order to detect the presence of bone or other hard tissues which may be present. Specimens one-half of an inch square should be taken from the periphery, center, and base of the tumor, and placed in a glass bottle in a 4 percent formalin solution as above, and shipped in accordance with the appended regulations.

URINE

The liquid should be placed in a thoroughly cleaned and dried bottle. The bottle must be tightly stoppered and should hold anywhere from 8 to 16 ounces. This should be surrounded by cracked ice and sawdust, and immediately shipped to the laboratory. A letter should accompany the specimen stating the nature of the examination required.

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APPENDIX

TRAFFIC RULES AND INSTRUCTIONS

(Issued July 1, 1915, cancelling "Traffic, Rules and Instructions" issued May 15, 1912, and all supplements thereto.)

5. Bodies or parts thereof or heads of dogs or other animals by express to laboratories.

(1) Rules and Regulations governing the shipments of the heads of dogs or other animals by express to laboratories of the State Board of Health or other laboratories,

(a) Agents must not accept for transportation the head of a dog or any other animal sent to the State Boards of Health for rabies examination unless it shall have been prepared for shipment as hereinafter provided.

(b) The head of a dog or other animal so shipped must be placed in a tin or other metal container, which will not permit the leakage of fluids; such container shall then be placed in a wooden or other container with ice packed around it. Such outside container must be so constructed that it will not permit the leakage of ice water.

(c) All such packages must be labeled: CAUTION—This package contains the head of a dog (or name of other animal) suspected of having died of hydrophobia.

(d) Such shipments tendered on Saturday, which cannot reach destination early enough for delivery on that day, and would, therefore, remain in the express office over Sunday, must be refused, and shipper requested to pack in ice and hold until Monday, so that they can be delivered without delay at destination.

(e) Require prepayment of charges on shipments of this kind.

(2) Dog and cat heads for the Pasteur Institute, Austin, Tex.

We are advised by the Superintendent of the Pasteur Institute of Austin, Tex., that shipments of dog and cat heads sent to the institute for the purpose of ascertaining if the animal before killed was rabid, are received in bad condition on account of inadequate packing and protection.

These shipments must be packed in ice sufficient in quantity to preserve until delivery. Charges must be prepaid. In addition to transportation charges to Austin, a fee of 50 cents is required for special delivery to the Institute, which is considerable distance out, and a special delivery is necessary in each case by a local deliveryman, whose charge is 50 cents.

(3) Bodies of dead animals or parts thereof.

Various State Boards of Health have decided that the custom of shipping bodies of dead animals (especially dogs) to state laboratories or other public or private laboratories is unnecessary, and a menace to the health of the people, and regulations have been established that whenever analysis of dead animal tissues becomes necessary, the brain, spinal cord, stomach, liver, or other part, organ, or tissue affected, MUST BE REMOVED FROM THE BODY OF THE ANIMAL BEFORE DELIVERING TO THE EXPRESS COMPANY, AND PACKED, ICED,

AND LABELED, AS PROVIDED ABOVE FOR "HEADS OF DOGS OR OTHER ANIMALS," IN PARAGRAPH 1. (*b, c, and d*). CHARGES MUST BE PREPAID.

Agents at all points are instructed to comply with these instructions, and to refuse shipments as above described unless packed as provided above.

**POSTAL LAWS AND REGULATIONS RELATING TO
DISEASED TISSUES**

(Section 473. Extract.)

When Mailable

Specimens of diseased tissues may be admitted to the mail for transmission to United States, state, municipal, or other laboratories in possession of permits referred to in paragraph 3 of this section only when inclosed in mailing cases constructed in accordance with this regulation, provided that bacteriologic or pathologic specimens of plague and cholera shall under no circumstances be admitted to the mails.

When Not Mailable

2. Liquid cultures, or cultures of microorganisms in media that are fluid at the ordinary temperature (below 45° C. or 113° F.), are unmailable. Such specimens may be sent in media that remain solid at ordinary temperature.

Permit from Postmaster General Before Delivery

3. No package containing diseased tissue shall be delivered to any representative of any of said laboratories until a permit shall have first been issued by the Postmaster General, certifying that said institution has been found to be entitled, in accordance with the requirements of this regulation, to receive such specimens.

Preparation for Mailing

4. (*a*) Specimens of tubercular sputum (whether disinfected with carbolic acid or not disinfected) shall be transmitted in a solid glass vial with a mouth not less than 1 inch in diameter and capacity of not more than 2 ounces, closed by a cork stopper or by a metallic screw top protected by a rubber or felt washer. Specimens of diphtheria, typhoid, or other infectious or communicable diseases or diseased tissues, shall be placed in a test tube made of tough glass, not over three-fourths of an inch in diameter and not over 7½ inches in length, closed with a stopper of rubber or cotton, and sealed with paraffin or covered with a tightly fitting rubber cap.

(*b*) The glass vial or tube shall then be placed in a cylindrical tin **box**, with soldered joints, closed by a metal screw cover with a rubber or felt washer. The vial or test tube in this tin box shall be completely and evenly surrounded by absorbent cotton closely packed.

(*c*) The tin box with its contents must then be inclosed in a closely fitting metal, wooden, or papier-mache block or tube, at least three-

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sixteenths of an inch thick in its thinnest part, of sufficient strength to resist rough handling and support the weight of the mails piled in bags. This last tube shall be tightly closed with a screw-top cover with sufficient screw threads to require at least one and one-half full turns before it will come off, and fitted with a felt or rubber washer.

Specimens for Malaria or Typhoid Fever Test

5. Specimens of blood dried on glass microscopic slides for the diagnosis of malaria or typhoid fever by the Widal test may be sent in any strong mailing case which is not liable to breakage or loss of specimen in transit.

Indorsement on Packages

6. Upon the outside of every package of diseased tissues admitted to the mails shall be written or printed the words "Specimen for bacteriological examination. This package to be pouched with letter mail."

