TB MED 237

DEPARTMENT OF THE ARMY TECHNICAL BULLETIN

COLLECTION AND PREPARATION OF SPECIMENS FOR SHIPMENT TO MEDICAL LABORATORIES

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1. GENERAL. This technical bulletin will serve as a guide for the collection and preparation for shipment of laboratory specimens. Changes in technics and methods will require modifications of these instructions and such modifications as may be indicated may be effected by directions furnished by individual laboratories to contributors. Care must be taken to prepare specimens properly, to label them accurately and to enclose the appropriate forms showing the pertinent clinical data and the type of laboratory examination desired. Specimens which may deteriorate at high temperatures should be held in refrigerator until shipped and should not be shipped so as to be delayed in the mails over a week end.

2. SPECIMENS FOR TOXICOLOGICAL ANALYSIS. *a.* Material for toxicological analysis will be sent to the appropriate army area medical laboratory if the services of a chemist are not available locally. The army area medical laboratory will forward specimens to the Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C., Attention: Chemistry section, if the examination cannot be performed at the army area medical laboratory or if it is desired that duplicate analysis be made. Containers will be chemically clean, accurately labeled and wrapped in heavy paper, with the edges of the paper secured by sealing wax bearing a distinctive device so that any tampering will be evident. Clinical data must accompany the specimen in order that the chemist may know what poisons are suspected. In the case of autopsy specimens the autopsy protocol will be included with the clinical history of the deceased. Tissues for toxicological analyses must not be placed in formalin but should be preserved by packing the containers in dry ice or ice. If it is not possible to preserve specimens with dry ice or ice they may be placed in ethyl alcohol providing analysis for alcohol is not desired. A specimen of the

alcohol used should be sent in a separate chemically clean container. Another preservative which may be used is sodium chloride, one part sodium chloride to three parts of tissue. A portion of the sodium chloride should be sent in a separate chemically clean container. See brochure entitled, "The Army Autopsy and Histopathological Service," available from the Armed Forces Institute of Pathology, Washington 25, D. C., and chapter 16, TM 8-227.

b. The following minimal amounts of each type of specimen should be forwarded to the laboratory. It is better to send too much specimen rather than too little.

(1) Bone	200 gm.
(2) Blood	25–50 ml.
(3) Brain	600 gm.
(4) Hair	10 gm. or more if possible.
(5) Intestinal contents.	100–150 gm.
(6) Kidney	One kidney.
(7) Liver	800 gm.
(8) Lung	One lung.
(9) Muscle	200 gm.
(10) Spinal fluid	All available.
(11) Stomach contents.	All available up to 1,000 ml.
(12) Urine	All available up to 1,000 ml.

3. SPECIMENS FOR BACTERIOLOGICAL EXAMINATIONS. Officers in charge of laboratories doing definitive bacteriology will forward to the Army Medical Service Graduate School cultures of such organisms as may be disignated from time to time by The Surgeon General.

a. Blood cultures. Prepare appropriate media in screw cap 120 ml. bottles (item No. 4–056–400) and add 0.5 ml. of blood per 10 ml. of media. Chances of a positive culture are increased if several bottles of media are inoculated. Ship in double mailing case (item No. 4–124–060). If the patient is receiving sulfa drugs, add to the media, before autoclaving, 0.005 percent para-aminobenzoic acid or, if the patient is receiving penicillin add one percent sterile penicillinase (Penase) to the sterile medium. (Penicillinase is thermolabile.) (Par. 270, *h* and 272, *i*, TM 8–227.) Enclose essential clinical data with all blood cultures.

b. Culture for identification or for testing antibiotic sensitivity. Send a pure culture of the organism in a double mailing case (item No. 4–124–020). Indicate the source of the organism.

c. Urine, cerebrospinal fluid, plueral, or other body fluids for culture. Collect specimens aseptically and inoculate appropriate liquid media or send the fluid in a sterile screw-cap vial (item No. 4–082–300). Some body fluids such as pleural fluid may coagulate. This can be prevented by collecting the fluid in sterile tubes containing 20 mg of potassium oxalate per 10 ml. of fluid.

d. Sputum of gastric washings for acid-fast organisms (smears, culture or guinea pig inoculation). Mix with an equal volume of 23 percent trisodium phosphate in a wide-mouth, screw-cap vial (item No. 4–089–150 or 4–089–185). Ship in a double mailing case (item No. 4–124–060) (par. 386, TM 8–227). Avoid delay.

e. Isolation of fungi from skin scrapings, sputum, body fluids, etc. Send material in sterile, wide-mouth, screw-cap vial or inoculate appropriate media. (Par. 399, TM 8–227.)

f. Feces for culture. Add a small portion of feces to sterile buffered glycerol-saline solution in a sterile, wide-mouth, screw-cap vial and ship in a double mailing container (item No. 4–124–060) (par. 231, *a*, TM 8–227).

g. Food poisoning outbreaks. Send samples of suspected food, vomitus and feces, frozen in dry ice in sterile containers such as wide-mouth, screw-cap bottles (item No. 4–089–150 or 4–089–185), or a sterile half pint fruit jar (item No. 4–306–000). Include essential clinical data.

h. Water for bacteriological examination. Use sterile, screw-cap 120 ml. bottles prepared by army area laboratories containing 0.02–0.05 gm. sodium thiosulfate. Ship in double mailing container (item No. 4–124–060). Such samples cannot be used for residual chlorine tests. (See TB Med 163 and TB Med 229.)

i. Milk or ice cream for bacteriological examination. Since milk, ice cream, buttermilk, etc., must be kept iced in shipment, it is best to do the examination locally if possible. (See TB Med 233, and par. 3, SR 40–920–1.)

j. Food specimens. See TB Med 233, and SR 40–920–1.

4. SPECIMENS FOR SEROLOGICAL EXAMINATION. *a. General.* Specimens of whole blood, serum or cerebrospinal fluid for serological tests which must be shipped should be kept sterile, especially in warm weather and when the specimen will be delayed more than 24 hours in transit. Use sterile dry syringes and needles in collecting blood specimens to avoid hemolysis. It is best to separate serum from the clot in order to avoid hemolysis. Merthiolate may be used to prevent bacterial growth in serum and does not interfere with most serological tests. Use 0.1 ml. of a 1–100 stock aqueous solution for each 5 ml. of serum. Do not add merthiolate to cerebrospinal fluid nor to specimens of serum for neutralization tests. Do not use *tincture* of merthiolate. Specimens to which merthiolate has been added should be so marked.

b. Detailed instructions.

(1) *Blood grouping.* Send 5 ml. sterile whole blood, clotted or oxalated.

(2) *Cross-match.* Send 5 ml. sterile whole blood, clotted or oxalated.

'3) *Rh factor.* Send 5 ml. sterile whole blood clotted or oxalated.

(4) *Rh antibody study.* Collect 10 ml. sterile whole clotted blood. Submit serum and clot separately to avoid hemolysis. Keep sterile.

(5) *Agglutination tests for typhoid, paratyphoid, brucellosis, tularemia, rickettsial diseases, heterophile antibody, etc.* It is best to separate serum from the clot prior to shipment to avoid hemolysis. Send 3–5 ml. of the serum. Keep sterile. (See SR 40–305–6.) Since the time of appearance of antibodies in the blood varies with different diseases it is urged that all requests show the date of onset of symptoms and the tentative diagnosis. It is always best to send two specimens, one taken early in the disease and one taken later so that a rise in titer can be demonstrated.

(6) *Cold agglutinins.* Separate serum from the clot before placing in refrigerator. If the whole blood has been inadvertently refrigerated, warm the blood in a 37° C. water bath for 15 minutes before removing serum. Keep sterile and submit 5 ml. of serum.

(7) *Cardiolipin microflocculation and complement-fixation tests.* Whole clotted blood (5 ml.) is satisfactory if time in transit is brief and specimen is not subjected to high temperatures. It is best to send sterile serum.

(8) Cerebrospinal fluid for colloidal gold determinations must be free of blood and should be kept sterile.

c. Blood specimens for other examinations as indicated below.

(1) *Blood for malaria.* Send thick and thin film. Fix the thin film in methyl alcohol. Do not fix the thick film.

(2) *Blood dyscrasia.* Send unstained blood smears and smears of sternal marrow.

5. CHEMICAL EXAMINATIONS OF BLOOD. (See sec. 1, ch. 6, TM 8–227.) *a. General.* Specimens of blood, plasma or serum for chemical analysis which must be shipped should be kept sterile. Plasma or serum should be separated from the blood or the protein-free filtrate prepared, within one hour after the blood is collected and specimens should be kept in the refrigerator pending analysis or shipment. Whenever possible, the local installation should prepare the protein-free filtrate rather than shipping whole oxalated blood since the protein-free filtrate keeps better than whole blood. If whole blood is shipped, 10 mg. of sodium fluoride per 10 ml. of blood will prevent coagulation and will prevent loss of sugar by glycolysis; it also appears to stabilize uric acid. Fluoride also acts as a bacteriostatic agent and is recommended when whole blood must be shipped for creatinine, nonprotein nitrogen or sugar analysis. It cannot be used if urea is to be determined since it inhibits the action of urease. One milligram of thymol per milliliter of blood may be added with the 10 mg. of fluoride to prevent bacterial growth. The above chemicals may be combined with potassium oxalate by mixing in a mortar 1 part of thymol, 3 parts of potassium oxalate and 10 parts of sodium flouride. Add 4 mg. of this mixture for each milliliter of blood, using a small spoon which will measure the correct amount. Blood can be preserved for a week with this mixture for examination for nonprotein nitrogen, uric acid, creatinine, and sugar. Dry sterile syringes and needles must be used in collecting blood. Water in the needle

or syringe will cause hemolysis. Avoid use of force and foaming in transferring blood from the syringe to the tube. (See par. 189, TM 8- 227.)

b. Anticoagulants.

(1) Potassium oxalate is readily soluble and is recommended as the anticoagulant for blood chemistry specimens. Prepare a 10 percent neutral potassium oxalate solution (adjust to ph 7.4 with phenol red indicator using oxalic acid) and pipette 0.1 ml. (0.01–0.02 ml. per ml. of blood to be received) into sterile tubes or flasks. Distribute in a thin film about the sides and bottom of the vessel and dry in the incubator or in an oven with a temperature of less than 80° C. Higher temperatures may decompose the oxalate into carbonate. Seal with rubber stoppers. The oxalate used provides 1 to 2 mg. per ml. of blood if 6 to 10 ml. of blood are added. Less oxalate may not prevent clotting and more oxalate may cause hemolysis; may alter the distribution of electrolytes, notably chlorides and bicarbonate, between cells and plasma; may also prevent proper tungstic acid precipitation of the proteins during the preparation of the protein-free filtrate and may cause clouding in nesslerizing.

(2) Lithium or sodium oxalate may be substituted for potassium oxalate but ammonium oxalate, because of its ammonia content, cannot be used if the analysis is based on the determination of ammonia such as in the Kjeldahl method for NPN. Lithium oxalate avoids the white precipitate which may occur in uric acid determinations when potassium oxalate is used.

(3) Heparin in the amount of 0.2 mg. per ml. of blood is an excellent anticoagulant for all purposes. It is the one anticoagulant which provides a plasma that can be used as well as serum for calcium determinations.

(4) Sodium citrate (5 mg. per ml.) can be used when whole blood is to be analyzed. When plasma is to be analyzed, oxalate is preferable as the citrate causes more disturbance in the distribution of water and electrolytes between cells and plasma.

(5) A mixture of ammonium and potassium oxalates has the advantage of not changing cell volume to any extent and is the preferred anticoagulant for hematocrit determinations or for the determination of plasma proteins by the copper sulfate gravity method. Dissolve 1.2 gm. of ammonium oxalate and 0.8 gm. of potassium oxalate in 100 ml. distilled water and use 0.1 ml. for each 1 ml. of blood.

(6) Sodium fluoride. (See *a* above.)

6. HANDLING SAMPLES OF WHOLE BLOOD FOR CHEMICAL ANALYSIS.

Analysis	Amount required	Can be shipped for analysis	Remarks
Alcohol	10 ml	Yes	100 mg. sodium fluoride per 10 ml. blood. Use sterile tube and keep tightly stoppered.
Barbiturates	10 ml	Yes	
Carbon monoxide	10 ml	Yes	Avoid escape of CO by placing in closed container with little air space.
Hemoglobin and hematocrit by copper sulfate method.	1 ml	Yes	Use ammonium and potassium oxalate as anticoagulant (par. 70, *c*, TM 8-227).
Sugar	5 ml	Yes	Use 10 mg. fluoride per ml. blood or the mixture of fluoride, thymol and potassium oxalate. Best to send protein-free filtrate.
Sulfonamides	5 ml	Yes	Specify sulfa drug.
Urea nitrogen	5 ml	Yes	Do not use fluoride; it inhibits urease action. Send protein-free filtrate or defibrinated sterile whole blood.

7. HANDLING SPECIMENS OF SERUM OR PLASMA FOR CHEMICAL ANALYSIS.

Analysis and material used	Amount required	Can be shipped for analysis	Remarks
Albumin, total protein, A/G ratio, plasma.	8 ml.	Yes	Avoid hemolysis.
Ascorbic acid, plasma	2 ml.	No	Avoid hemolysis. Do not separate plasma till ready to do test as ascorbic acid deteriorates faster in plasma than in whole blood. Do test within 30 minutes.
Amylase, serum (or plasma)	5 ml.	No	Do test within 1–2 hours. Avoid hemolysis.
Bilirubin, (van den Bergh) serum	5 ml.	No	Avoid hemolysis. Do test within 2 hours.
Bromides, serum	7 ml.	Yes	
Bromsulfalein, serum	3 ml.	Yes	Avoid hemolysis.
Calcium, serum	5 ml.	Yes	Separate serum at once and add 1 drop of toluene for shipping. Use acid-cleaned tube. Avoid hemolysis.
Cephalin flocculation, serum	5 ml.	No	Do test within 4 hours. Avoid hemolysis.
CO_2 combining power, plasma or serum.	5 ml.	No	Avoid venous stasis in collecting blood. Avoid hemolysis. Use neutral oxalate. Separate plasma or serum promptly. Avoiding any unnecessary agitation of the whole blood.
Chloride	5 ml.	Yes	Avoid venous stasis in collecting blood. Avoid excess oxalate and avoid hemolysis. Separate serum or plasma at once and without undue agitation.
Cholesterol total, plasma or serum	5 ml.	Yes	Separate plasma or serum promptly. Avoid hemolysis.
Cholesterol esters, plasma or serum.	5 ml.	No	Plasma or serum may be kept 1–2 days in refrigerator but only 1–2 hours at room temperature. Avoid hemolysis.
Creatinine, plasma	5 ml.	Yes	
Fatty acids, plasma or serum	5 ml.	Yes	
Fibrinogen, plasma	5 ml.	Yes	Use 2 mg. potassium oxalate per ml. blood. Avoid any agitation which may cause hemolysis with consequent high values. Separate plasma before mailing.
Icterus index, serum	3 ml.	Yes	Avoid hemolysis. Keep sterile.
Lipase, serum	5 ml.	No	Do test within 1–2 hours.
Phosphorous, serum preferred because less danger of hemolysis than with plasma.	5 ml.	Yes	Avoid hemolysis and delay in separating serum (or plasma). Use acid-cleaned tube. Add one drop of toluene to the serum (or plasma) for shipping. Best to ship the trichloracetic acid filtrate.
Phosphatase, alkaline or acid, serum.	10 ml.	No	Separate serum at once and place in refrigerator. Avoid hemolysis. Will keep one hour at room temperature and several hours in the refrigerator. After 24 hours phosphatase increases 10–20 percent.
Potassium, serum	10 ml.	Yes	Avoid hemolysis. Separate serum at once and keep in refrigerator while in the laboratory. Use acid-cleaned tube and waxed cork.
Proteins by copper sulfate method, plasma or serum.	1–5 ml.	Yes	Use ammonium and potassium oxalate mixture as anticoagulant. Par. 11, *b*, (5). Avoid hemolysis.
Salicylates, serum or plasma	10 ml.	Yes	
Sodium, serum	3–5 ml.	Yes	Avoid hemolysis. Separate serum at once. Use acid-cleaned tube and waxed cork.
Thymol turbidity, serum	5 ml.	Yes	
Uric acid, plasma or serum	5 ml.	Yes	Substances such as ergothioneine and gluthathione which give the same color reaction are largely eliminated by using serum rather than whole blood.

8. CHEMICAL ANALYSIS OF WATER. Send one gallon of water in a chemically clean, glass-stoppered bottle, item No. 4–054–000.

9. EXAMINATION OF URINE. *a. Chemical.*

(1) Toluene is a satisfactory preservative for most chemical examinations. Use only a few drops, sufficient to form a thin layer over the surface.

(2) For 17-ketosteroids collect a 24-hour specimen of urine directly into a chemically clean, glass-stoppered bottle containing 10 ml. of concentrated HCL, *use no toluene or chloroform.* Send at least 300 ml. of this specimen to the laboratory with a notation as to the total 24-hour amount. An abstract of the clinical record should accompany the specimen.

(3) For lead collect a 24-hour specimen and submit at least one-third the 24-hour amount with a notation as to the total 24-hour volume. The containers used for collecting and shipping the specimen must be lead free. Pyrex flasks may be rendered lead free by boiling with chemically pure nitric acid. Add a small amount of nitric acid and bring to a boil under a hood. Rinse the walls by tilting the flask and let stand one hour. Rinse again with the nitric acid and then rinse with distilled water several times. Keep stoppered at all times.

b. Formed elements in the urine. Add 2 drops of formalin (40 percent formaldehyde) per 100 ml. of urine if the specimen is to be shipped. Do not use more formalin since larger amounts interfere with tests for sugar, albumin and indican.

c. Friedman test. Submit at least 100 ml. of the first morning specimen in a clear (preferably sterile) screw-cap bottle. If the specimen is to be mailed add one drop of tricresol or 0.4 gm. of boric acid crystals for each 25 ml. of urine. Patients should be instructed to drink very little water the evening before the test and not to take any aspirin, barbiturates or quinine as these drugs will kill the rabbit.

d. Aschheim-Zondek test. For the quantitative Aschheim-Zondek test, collect a 24-hour specimen in a clean bottle. Note the 24-hour volume on the request form and submit at least 250 ml. If the specimen is to be mailed add one drop of tricresol or 0.4 gm. of boric acid crystals to 25 ml. of urine.

10. VIROLOGY SPECIMENS. *a. General.* A complete diagnostic service for virus and rickettsial diseases is available only at the Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C. Some army area medical laboratories and oversea laboratories are prepared to perform certain of these procedures. All specimens may be sent to the appropriate army area or oversea medical laboratory and that laboratory will forward such specimens as it is unable to examine, to the Army Medical Service Graduate School. Specimens may be sent direct to the school when it is known that the area or oversea laboratory is unable to perform the test desired. Area and oversea laboratories will notify contributing stations of the type of tests which must be sent direct to the school. It is important that specimens for the diagnosis of virus or rickettsial diseases be accompanied by an abstract of the clinical record in order that the laboratory may be guided in its selection of procedures. (See ch. 11, TM 8–227, TB Med 212, and SR 40–305–10).

b. Serological specimens for diagnosis of virus and rickettsial diseases. Most virus diseases can be identified by serological tests such as the complement-fixation test, neutralization test, Weil-Felix test, etc. This is a much simpler procedure than attempting to isolate and identify the virus itself. Two specimens of serum are usually required, one taken early in the disease and the other taken during convalescence. Diagnosis depends upon the demonstration of a rise in antibody titer in the "convalescent" serum as compared with the "acute" serum. The "early" or "acute" specimen should be collected as soon as possible after the onset of the disease and not later than the day indicated in *c* below. The "late" or "convalescent" specimen should be collected not sooner than the day indicated. Either whole blood or serum may be submitted but serum is preferable. Specimens should be kept sterile. The serum should be separated if the specimen will be subject to sustained temperatures of over 100° F. in transit. Vacuum tubes are convenient for collecting and shipping whole blood. At least 5 ml. of serum or 10 ml. of whole blood should be sent. Specimens should not be frozen and need

not be refrigerated in transit but should be kept in the refrigerator while in the laboratory. In the case of suspected influenza, it is best to hold the "acute" specimen until the "convalescent" specimen is obtained and to send both at the same time. In the case of other diseases, send the "acute" specimen and follow with the "convalescent" specimen. Label both specimens using adhesive tape or a paper label covered by transparent tape. The label must show the patient's name, service number, grade, organization, station and date of collection. An abstract of the clinical record, including laboratory data, must accompany the specimens. Progress notes should accompany "convalescent" specimens. Use air mail if ordinary mail requires more than 24 hours. Label the package "Specimen for Virus Diagnosis. Rush. Keep cool," and send to the appropriate army area medical laboratory or to the Department of Virus and Rickettsial Diseases, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.

c. Serological methods available for virus and rickettsial diseases.

Disease	Test	Day of bleeding	
		Early specimen—Before	Late specimen—After
Virus:			
Atypical pneumonia	Cold hemagglutinins	7	21
Colorado tick fever	Complement fixation or neutralization test	6	21
Dengue fever	Neutralization test	6	21
Encephalitides:			
Eastern or western equine	Complement fixation or neutralization test	6	21
Japanese	Complement fixation or neutralization test	6	21
Russian spring	Complement fixation or neutralization test	6	21
Russian summer		6	35
St. Louis	Complement fixation or neutralization test	6	21
Venezuelan	Neutralization test	6	21
West Nile	Neutralization test	6	21
Herpetic:			
Herpes simplex	Neutralization test	6	21
Influenza (see par. 3 SR 40-210-20)	Red cell agglutination-inhibition	2	8
Lymphocytic choriomeningitis	Complement fixation	10	21
	or		
	Neutralization test	21	42
Lymphogranuloma venereum	Complement fixation	10	21
Mumps meningitis	Complement fixation	6	21
Psittacosis	Complement fixation	10	21
Rift Valley fever	Neutralization test	6	21
Yellow fever	Complement fixation or neutralization test	6	21
Rickettsial Diseases:			
Epidemic Typhus	Complement Fixation	10	21
	Rickettsial agglutination	6	15
	Weil-Felix, OX-19, OX-2	6	12
Fievre boutonneuse	Complement fixation	10	21
	Weil-Felix OX-19	6	12
Murine Typhus	Complement fixation	10	21
	Rickettsial agglutination	6	15
	Weil-Felix OX-19, OX-2	6	12
"Q" fever	Complement fixation	10	21
Rickettsialpox	Complement fixation	10	21
Rocky Mountain spotted fever	Complement fixation	10	21
	Weil-Felix, OX-19, OX-2	6	12
Scrub Typhus	Weil-Felix, OX-K	6	12
South African tick fever	Complement fixation	10	21
	Weil-Felix OX-19	6	12

d. Isolation and identification of virus and rickettsial agents.

(1) The isolation and identification of virus and rickettsial agents is a laborious and time-consuming procedure which is seldom practical. Isolation procedures should be limited to the following conditions:

(*a*) From brain tissue obtained at necropsy in cases with a clinical diagnosis of encephalitis, aseptic meningitis or rickettsial disease.

(*b*) From cerebrospinal fluid or blood collected during the *early* febrile period in cases of suspected lymphocytic chorio-meningitis.

(*c*) From cerebrospinal fluid collected during the *early* febrile period of meningitis associated with lymphogranuloma venereum.

(*d*) From lung tissue obtained at necropsy from a case of influenza or atypical pneumonia.

(*e*) From various organs obtained at necropsy if an outbreak of a fatal disease of unknown etiology occurs.

(2) Specimens of serum for antibody studies must be submitted on all living patients from whom materials are sent for isolation of virus.

(3) Specimens submitted for isolation procedures must be frozen immediately on collection and shipped in sufficient dry ice to insure their arrival in the frozen state. Use 10 pounds of dry ice for a shipping time of 24 to 36 hours. If the specimen is serum or cerebrospinal fluid place in a sterile thick wall tube or vial and freeze by swirling in a mixture of dry ice and alcohol. Do not freeze whole blood but separate and freeze the serum. Keep specimens sterile. Use rubber stoppers fastened with adhesive tape. Wrap the tube in cotton and place in a metal mailing case. Place the mailing case in a cardboard box with the dry ice and place this box in a larger box which acts as insulation. Use newspapers or sawdust as packing material in both boxes and wrap both in heavy paper. A vacuum bottle may be used if available. Wrap the tube in cotton and place in the vacuum bottle with crushed dry ice. *Do not touch dry ice with bare hands. Use forceps or gloves.* The cork of the vacuum bottle must be notched to permit escape of CO_2. Pack the vacuum bottle in a cardboard box. Specimens of tissue are frozen in wide-mouth sterile bottles. Ship by air express or air mail, special delivery to the Department of Virus and Rickettsial Diseases, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C. Telegraph the School when the specimen is sent so its handling can be expedited. Label the package "Rush! Keep cool!"

(4) (*a*) Specimens of tissue may be preserved in 50 percent buffered glycerin if dry ice is not available or if shipment requires more than 36 hours. Use a block of tissue about 1 cm. in thickness and 2 cm. square in 100 ml. of 50 percent buffered glycerin in a wide-mouth sterile bottle. Extreme care must be exercised to assure that the bottle used can be tightly closed and sealed to prevent leakage of the buffered glycerin.

(*b*) The 50 percent buffered glycerin is prepared as follows: Prepare 2.1 percent citric acid and 2.84 percent anhydrous Na_2HPO_4 in double distilled water. To make 100 ml. of buffer use 9.2 ml. of the citric acid solution and 90.8 ml. of the Na_2HPO_4. This mixture should have a pH of 7.4. Half fill wide-mouth cotton stoppered bottles with equal parts of the buffer and C. P. glycerin and sterilize at 15 pounds pressure for 30 minutes. Replace cotton topper with sterile rubber stopper.

(*c*) In emergencies, freshly boiled double distilled water may be substituted for the buffer solution in preparing the 50 percent glycerin.

(5) The specimens to be submitted for isolation procedures in various virus and rickettsial diseases are listed below. Preferably all specimens should be frozen.

Blocks of tissue should be about 1.0 cm. in thickness and 2–4 cm. square. Isolation procedures are to be discouraged, except as enumerated in (1) above.

Disease suspected	Material to be submitted for isolation of agent	
	Clinical	Autopsy
Colorado tick fever	Blood.	
Coxsackie group	Feces or nasal washings.	
Dengue fever	Blood.	
Encephalitis:		
Equine	Blood	Brain.
Japanese	Blood	Brain.
Russian spring-summer	Blood Cerebrospinal fluid (early)	Brain.
St. Louis	Blood (agent rarely isolated)	Brain.
West Nile	Blood	Brain.
Epidemic keratoconjunctivitis	Conjunctival secretions.	
Fort Bragg or Pretibial fever	Blood.	
Herpes simplex	Vesicle, cerebrospinal fluid	Brain, cerebrospinal fluid.
Influenza	Nasal and throat washings	Lung.
Lymphocytic choriomeningitis	Blood, cerebrospinal fluid	Brain.
Lymphogranuloma venereum	Cerebrospinal fluid, Pus from bubo	Brain.
Mumps	Saliva, cerebrospinal fluid.	
Phlebotomus (sandfly) fever	Blood.	
Poliomyelitis	Feces	Spinal cord, medulla.
Psittacosis	Sputum, blood	Spleen, lung, blood.
"Q" fever	Blood or urine.	
Rabies	Saliva	Brain. In case of suspected rabies in animals, send the whole head packed in wet ice or dry ice or send a portion of hippocampus major in buffered glycerin. For demonstration of Negri bodies send whole head packed in wet ice in an insulated container. Do not use dry ice as it causes rupture of cells.
Rickettsialpox	Blood.	
Rocky Mountain spotted fever	Blood	Spleen, brain, blood.
Scrub typhus	Blood	Spleen.
Typhus, epidemic or murine	Blood	Spleen, liver, blood.
Variola	Vesicle fluid or crusts	Vesicle fluid or crusts.
Yellow fever	Blood	Liver, brain.

11. PARASITOLOGICAL SPECIMENS.

a. Feces. Stool specimens may be shipped in a 60-ml. wide-mouth, screw-top bottle in a double mailing container. The bottle should not be filled more than half full. Specimens containing oil are unsatisfactory. If the specimen is to be preserved, emulsify a portion in water and add an equal volume of 10 percent formalin.

b. Fecal smears for protozoan trophozoites. Fecal smears should be fixed in Schaudinn's solution and shipped in 70 percent alcohol in a wide-mouth, screw-top bottle with sufficient cotton to protect the slides. Use a double mailing container (par. 456, TM 8-227).

c. Helminths. Helminths such as nematodes, flukes or cestodes may be preserved in 10 to 20 volumes of 5 percent formalin. Flukes and cestodes should be placed on stiff paper or cardboard to preserve the shape.

d. Smears for malaria, microfilaria, leishmania, trypanosomes, etc. Ship unstained slides by wrapping each slide in several thicknesses of toilet paper, tying the group of slides together and wrapping or packing in cotton. Both thick and thin films should be submitted for malaria.

12. ENTOMOLOGICAL SPECIMENS.

a. General. Insects and other arthropods of medi-

cal or economic importance, especially all species of mosquitoes and disease vectors, will be collected, whenever possible, in order that control measures can be adapted to the species concerned and that representative collections may be available from all geographical areas where troops are stationed. Complete data for each lot of specimens including date, locality, elevation, host, habitat, name of collector (and, when indicated, epidemiologic data of the regions) will accompany the shipments. Medical laboratories are authorized to distribute to post surgeons such supplies as may be necessary for the transmission of specimens to the appropriate medical laboratories. Reports will be returned by laboratories to the medical officer who forwarded the specimens. Transfer of the information contained in these reports to other agencies for the best interests of military service is authorized at the discretion of the post surgeon, unless otherwise directed by the responsible commanding officer. Specimens which cannot be identified by the area medical laboratory, or which are considered of sufficient significance for museum use, will be forwarded to the Army Medical Service Graduate School for further study and disposition.

b. Mosquito larvae.

(1) *Collection and preservation.* Collect a series of fully grown larvae of each species in an area, and preserve in 70 percent alcohol. Kill in any manner that will prevent distortion and discoloration. A convenient method is to drop them in hot water (not boiling) for 10 to 20 seconds; then transfer to 50 percent and finally 70 percent alcohol.

(2) *Packing.* Avoid injury to larvae by placing them in a small vial or glass tubing filled with alcohol. Exclude all air bubbles. Plug vial with cotton, and then place it in a larger vial filled with alcohol. A small air bubble should be present in the larger container to allow for expansion. Any number of larvae may be placed in the small vial as long as the specimens do not become crushed. Place in the large vial a slip of paper on which pertinent data have been written with lead pencil.

c. Mosquito adults.

(1) *Collection and preservation.* Mosquito adults are very delicate and must be handled carefully to avoid loss of scales or appendages. Moisture will cause scales to lose their natural color. Condensation of moisture on the inside of a chloroform collecting tube occurs quickly if the tube is left in the sun or a heated place. Mosquitoes captured in a killing tube should be removed as soon as killed. Specimens will also be ruined if left too long in a relaxing jar for softening. Reared specimens should be kept alive 12 hours to allow them to harden.

(2) *Packing.* Adult mosquitoes may be packed in pill boxes between layers of cellu-cotton or lens paper. Toilet tissue tends to rub off scales but may be used if it is the only material available. Cotton must not be used in contact with the specimens. The boxes should be kept in a larger box containing naphthalene and 1 to 10 percent DDT powder to protect against insects and molds but this material should not be in contact with the specimens. Label each pill box indicating date and place of collection.

d. Other arthropods such as fleas, flies, mites and ticks.

(1) Particular effort should be made to collect ectoparasites from wild rodents suspected of being reservoirs of disease. Unless the animal host is being prepared for the museum, the ears should be excised near the base of the skull and dropped into a dry vial. After eight hours fill the vial with 70 percent alcohol. This will preserve both the ears and the ectoparasites that have left the ears.

(2) Since fleas leave the animal after death it is best to capture the animal alive, kill it with chloroform or ether and place it in a paper bag where fleas, mites, lice and ticks may be collected. A pledget of cotton saturated with ether or chloroform may be placed in the bag to anesthetize the insects. Or the fur may be brushed with cotton moistened with ether or chloroform and the anesthetized fleas, lice, ticks and mites combed out on white paper. Insect powder such as DDT may also be used to kill the insects prior to combing the fur. Fleas may be collected

by combing the fur over water. The fleas will jump to the water where they can be easily collected. Place specimens in 70 percent alcohol for shipment.

(3) Lice may be picked from clothing and bedding with forceps or combed from hair with a fine tooth comb. If lice are to be preserved for identification, they should be kept alive till after digesting the blood meal. Place specimens in 70 percent alcohol for shipment.

(4) Parasitic mites may be collected by scraping the skin or in the case of dead animals portions of diseased skin may be removed and preserved. Place specimens in 70 percent alcohol for shipment.

(5) Ticks may be collected from their host by examination of all parts of the animal particularly around and in the ears, back of the head, above the root of the tail and inside the flanks. Care must be taken in removing ticks in order that the mouth parts do not break off and remain imbedded in the skin of the host. Chloroform will usually cause ticks to withdraw the capitulum. Ticks may be collected from vegetation by drawing a piece of white canton flannel over the area. Certain ticks (Argasidae) are collected in the habitats of their hosts, whereas some of the *Ornithodores* species bury themselves in the soil and can be collected by sifting soil over white paper. Place specimens in 70 percent alcohol for shipment.

(6) Sand flies are collected and handled for shipment in the same manner as mosquitoes. Sand flies are found in dark corners of dwellings or stables.

(7) All flies, except for delicate forms such as mosquitoes, sand flies, and gnats may be shipped in 70 percent alcohol.

13. MAMMALS. *a. Specimens.* Specimens of mammals should be collected whenever possible so that adequate collections from areas where troops are stationed will be available for study. The mammalian faunas especially of North Africa, Asia Minor, India, East Indian and Pacific Islands, and South America are inadequately represented in collections that are consulted. Additional material from other areas also would be of value. Specimens of mammals, particularly rodents of medical importance, which have not been recognized previously as significant in the area where collected and which are assumed or known to be involved in transmission of disease, including specimens suitable for museum display as well as those requiring identification or study, will be forwarded to the Armed Forces Institute of Pathology (ATTN: Director, Division of Mammals, U.S. National Museum, Washington, D. C.). All such packages should be marked "Skins of Mammals." For correct identification of species it is important to have skin and skull, to each of which should be attached a label showing:

(1) Number assigned to specimen.
(2) Sex.
(3) Locality where collected.
(4) Name of collector.
(5) Date.
(6) Total length (tip of nose to tip of tail vertebrae).
(7) Length of tail.
(8) Length of hind foot.

b. Collection and preservation. Skins of small and even of larger mammals may be removed with a pocket knife if no other instrument is available. Care should be taken not to damage the skull when the head is severed from the neck. Powdered arsenic and powdered alum dusted on the flesh side are recommended as the best preservatives for skins of small mammals. When cotton or tow is not available, paper folded to the proper size will make a satisfactory artificial body for the skin. Skins of mammals larger than rabbits should be salted and dried thoroughly. For more detailed instructions, consult "A Field Collector's Manual in Natural History," available from the Smithsonian Institute, Washington 25, D. C.

c. Packing. Prepared skins for study should be wrapped individually with paper and packed carefully to avoid distortion. Skulls should be packed in a separate tight container when placed in the package with skins, as insect larvae bred in the skulls may ruin skins in transit. Packages forwarded as franked mail should be wrapped securely and where materials are available should be sealed with glue, gummed paper, or adhesive tape. Furs, hides or skins of wild animals may be accepted for mailing only when properly dried or cured and plainly and clearly marked, labeled or tagged on the outside of the package with the

name and address of the shipper and consignee (addressee), together with such other indorsement, if any, as may be required by the game laws of the State, Territory, or District in which mailed. Parcels must be wrapped as to prevent grease soaking through the package and damaging other mail matter (sec. 35.18 (j) and 35.21, Postal Laws and Regulations, 1948).

d. Separate Data. When, for reasons of security, it is necessary to forward separately data relative to collected specimens, care should be exercised to attach tags, which are numbered according to items on the forwarded list, to the skin and skull of each mammal.

14. VETERINARY SPECIMENS. Directions for the collection and shipment of veterinary specimens are given in SR 40–920–1.

[AG 700.6 (19 Feb 52)]

BY ORDER OF THE SECRETARY OF THE ARMY:

J. LAWTON COLLINS
Chief of Staff, United States Army

OFFICIAL:
WM. E. BERGIN
Major General, USA
The Adjutant General

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NG: None.
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