



VIRAL AND RICKETTSIAL DISEASE LABORATORY

SPECIMEN PROCESSING MANUAL

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GENERAL INFORMATION

This Procedure Manual provides general information as well as specific instructions for processing clinical samples and performing support functions in the Immunoserology Section. Supporting Manual include:

- Specimen Receiving Room Staff Procedure Manual
- Viral and Rickettsial Disease Laboratory – Guidelines for Laboratory Services
- VRDL Safety Manual.

Commonly Used Abbreviations

COMMONLY USED ABBREVIATIONS			
<i>term</i>	<i>definition</i>	<i>term</i>	<i>definition</i>
VRDL	= California Department of Public Health Viral and Rickettsial Disease Laboratory	pt-pack	(Patient pack) = all submittal forms, lab results and communications relating to an individual patient medical record.
DCDC	= California Department of Public Health Division of Communicable Disease Control	Orange card	= 4 x 6 orange colored card used to indicate sample is being sequestered pending medical review.
MDL	= California Department of Public Health Microbial Disease Laboratory	LT	= laboratory technician
LCS	= California Department of Public Health Laboratory Central Services	LA	= laboratory assistant (includes both Senior Laboratory Assistant and Laboratory Assistant classifications)
LAU	= Laboratory Assistance Unit (previously known as the Medical Records and Local Assistance Unit)	RFFIT	= neutralizing test for rabies antibody
VBDB	= California Department of Public Health Vector Borne Diseases Branch	PCR	= polymerase chain reaction
CHD	= Local County Health Department or Local City Health Department	IF	= immunofluorescence assay (can be used for antibody or antigen detection)
PHL	= Local County Public Health Laboratory	NT	= neutralization assay
HO	= Local Health Officer	IgG	= immunoglobulin G
		IgM	= immunoglobulin M
SERO	= Serology Diagnostic Unit of VRDL		
ISOL	= Isolation Diagnostic Unit of VRDL		
Retro	= Retrovirus Diagnostic Unit of VRDL		

Types of Requests by Causative Agents

The Specimen Receiving Room staff has instructions to send any specimens with requests for tests on any of these agents listed below to the VRDL

Adenoviruses	Mumps virus
Anaplasma phagocytophilia	Mycoplasma species
Anti-HAV-IgM or IgG	Mycoplasma pneumoniae
Anti-HBc Ag	Newcastle
Anti-HBe Ag	Norovirus (Norwalk agent)
Anti-HBs Ag	Orf
Arboviruses	Parainfluenza 1, 2, 3, 4
Bedsonia	Parvovirus
California encephalitis (CEV)	Poliomyelitis (Polio)
Chikungunya (CHIK)	Psittacosis
Chlamydia	Q fever
Colorado tick fever (CTF)	Rabies
Coxiella burneti (Q fever)	Respiratory syncytial (RS)
Coxsackieviruses types A & B	Rhinoviruses
Cytomegalovirus (CMV)	Rickettsial pox
Dengue types 1, 2, 3, 4	Rocky Mountain Spotted Fever (RMSF)
Eastern equine encephalitis (EEE)	Rotavirus
ECHO Viruses	Rubella (German measles)
Enteroviruses	Rubeola (measles)
Epstein-Barr Virus	Smallpox
Ehrlichia sp. / E. chaffeensis	Small Round Structured Virus (SRSV)
Hantavirus	SLE (St. Louis encephalitis)
Hepatitis A (HAV)	Trachomatis
Hepatitis B (HBc Ag, HBe Ag, HBs Ag)	TORCH (Toxoplasma, Rubella, CMV, HSV1, HSV2, Herpes)
Herpes simplex virus 1 & 2, (HSV1, HSV2)	Typhus
Herpes Zoster (shingles)	Vaccinia
Human Immunodeficiency Virus (HIV)	Varcella (Chickenpox)
Human T Cell Lymphotropic Virus (HTLV)	Varicella-zoster (VZV) (shingles)
Influenza A & B	Variola
Japanese B encephalitis	WEE (Western equine encephalomyelitis)
Lymphocytic choriomeningitis (LCM)	West Nile Virus (WNV)
Lymphogranuloma venereum (LGV)	
Measles	
Milker's nodules	

Types of Requests by Disease Suspected

The VRDL will accept requests for the following diseases and syndromes:

Acute Respiratory Disease Syndrome (ARDS)	Kawasaki disease
Acquired Immune Deficiency Syndrome (AIDS)	Ornithosis
ALS (Amyotrophic Lateral Sclerosis)	Parotitis
Aseptic meningitis	Psittacosis (Parrot fever)
Chickenpox	Primary atypical pneumonia
Dawson's encephalitis	Q fever
Flu (or Influenza)	Reiter's syndrome
Guillain-Barre Syndrome	Reye's syndrome
Herpangina	Subacute sclerosing panencephalitis (SSPE)
Hantavirus Pulmonary Syndrome (HPS)	Tick fever

Types of Requests by Symptoms

If these requests are on VRDL submittal forms, route to VRDL; if on MDL forms, route to MDL. .

Acute Respiratory Disease Syndrome (ARDS)	Fever of unknown origin (FUO)
Bronchitis	Gastroenteritis
Central nervous system (CNS)	Hepatosplenomegaly
Conjunctivitis	Hepatitis
Encephalitis	Infectious mononucleosis
Meningitis	Pleurisy
Myocarditis	Pleurodynia
Neuritis	Pneumonia
Orchitis	Upper Respiratory Infection (URI)
Pericarditis	

Types of Specimens

Blood or serum	Vesicular lesion scraping (slide)
Other body fluids	Animal brain smears (rabies)
Feces (stool)	Autopsy or biopsy
Rectal swab (RS)	Brain, human or animal
Throat wash (TW)	cerebral cortex
Nasal-pharyngeal swab or wash (NP)	cerebellum
Spinal fluid (sp. fl.) (CSF)	meninges
Throat swab (TS)	mid-brain
Urine	temporal lobe
Vesicular fluid (lesion swab)	Bronchus
Amniotic fluid	Colon (intestines)
Isolates for identification	Fetus
Infected tissue culture fluid	Gut
Infected mouse brain suspension	Intestine
Animals	Kidney
Brain (for rabies)	Liver
Birds (psittacosis)	Lung
Mosquitoes	Nodes cervical, inguinal, mesenteric
Ticks	Pancreas
Slides containing clinical samples	Placenta
	Spinal cord
	Trachea

PROCESSING CLINICAL SAMPLES

Delivery of Samples

All specimens received for the VRDL are checked in by the Specimen Receiving Room Staff in Room 125 per instructions in the "Receiving Room Staff Procedure Manual for VRDL Specimens".

Samples are usually delivered at 10:00, 11:30 AM, 1:30, 3:30 and 4:30 PM. The Specimen Receiving Room Staff or the Laboratory/Senior Laboratory Assistant (LA) will call the LAU unit when samples are delivered.

The MRU staff may also request that a special delivery be made for specimens that require immediate handling. Alternatively for samples requiring immediate testing, MRU staff may go the Specimen Receiving Room and pick up the samples, check them in and deliver them directly to the testing unit.

All clinical samples for VRDL are delivered to the specimen receiving area of Room B335 with the following two exceptions:

- animal brains for rabies testing
- bat carcass for speciation that are delivered to Isolation walk-in refrigerator in Room B348.

Verification of Information.

 **All specimens should be handled using the universal precautions described in the VRDL Safety Manual.**

LAU staff are responsible for verifying that the information on patient samples brought to Room B335 match the submittal form. For improved QC, whenever possible, a second person, (usually a laboratory assistant) will assist in information verification. For the purpose of documentation, LAU staff will use blue pencil and laboratory assistants will use red pencil to put a "tick" (-) mark next to each verified piece of information. Information that is missing or incorrect on the submittal form will be corrected in blue or red pencil.

Verification is accomplished as follows:

- LAU staff will read aloud the laboratory accession number and name typed on the label attached by the Receiving Room Staff and verify that the name on the label matches the name written on the vial by the submitter. The laboratory assistant will place a "tick" mark when information agrees and inform LAU if there is disagreement.
- Continue the above procedure to verify the sample type and date collected from information on the sample.
- If the laboratory assistant is not available, LAU staff will verify that the information matches and put a blue "tick" mark on the submittal form whenever there is agreement.
- The laboratory assistant should call the LAU staff's attention to any form in which the patient information is not legible and print the information clearly in red pencil..
- LAU staff (or lab assistant) should print the patient's name in blue or red pencil if it is not readily legible on the form.

Guidelines for Screening Incoming Samples

Samples to be Sequestered Pending Medical Review

Samples with travel history (or test requests for agents suggestive of travel) to Asia, Africa and South America and Unusual Test Requests for agents not endemic to California will be sequestered pending medical review.

Examples of agents that must be sequestered pending Medical Review:

- Chikungunya, Dengue or Avian Influenza (suggesting travel to Asia)
- Flaviviridae (specifically - Absettarov, Central European tick-borne encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest, Omsk hemorrhagic fever, and Russian spring-summer encephalitis);
- Bunyaviridae (specifically – Congo-Crimean Hemorrhagic fever);
- Filoviridae (specifically – Ebola virus and Marburg virus);
- Arenavirus (specifically – Guanarito virus, Junin virus, Lassa Fever virus and Machupo virus);
- Herpesvirus simiae B;
- Tick-borne orthomyxoviridae.

Procedure for sequestering samples pending Medical Review

- Storage of Samples
 - a. Serums to be held in specially marked container in Room B335
 - b. Frozen respiratory samples to be held in specially marked container in freezer in Room B337
 - c. Refrigerated respiratory samples to be held in specially marked container on the middle shelf of the refrigerator in Room B335
- Originate an Orange form and attach to the Original Submittal form
- Put originals of submittal forms in basket in Dr. Louie's office –should Dr. Louie out of the office, Dr. Glaser (or Shilpa) and/or Dr. Schnurr can review.
- Following Medical Review, if the sample is to be released for testing, the Orange form will be so marked and returned so the sample can be placed back into general circulation.
- When the sample has been returned to general circulation, check the box on the Orange form and send back to the appropriate MR Unit. This Orange form will remain with the patient's pack as documentation that MR has been performed.

Saliva and CSF samples for suspected human rabies cases

Procedure for sequestering saliva samples pending Medical Review

- Frozen saliva and CSF samples to be held in specially marked container in freezer in Room B381
- Refrigerated saliva and CSF samples to be held in specially marked container on the lower shelf of the refrigerator in Room B381

 **Note for LAU Staff - Put originals of submittal forms in basket on Shilpa's desk marked "Hold Pending Medical Review".**

Samples requiring special handling

HEPATITIS C REQUESTS – Samples must be frozen if they cannot be tested within 72 hours.

- Freeze (or keep frozen) all samples for Hepatitis C testing.
- Store samples in the designated box in the freezer in room B-335
- Samples will be thawed prior to testing and when testing is completed, they will be returned to Denise for routine processing and storage at 4⁰C.

WHOLE BLOODS with ANTICOAGULANTS (purple, yellow or green tops)

Whole blood specimens are sometimes requested for speciality tests – such as EDTA (purple top) bloods for HPS PCR.

- Store these samples in the 4⁰C refrigerator in Room B335 until it is determined if it is appropriate to test them.

SAMPLES BROKEN or LEAKED in TRANSIT

- The lab assistant acts as the clean person per safety manual instructions.
- Wearing gloves and working in the BSC, LAU staff will place any salvageable forms in separate plastic bag held open by the lab assistant.
- Next LAU staff will open the specimen container. If it appears that the sample can be salvaged he/she will remove it using forceps and put it into a clean plastic bag held open by the lab assistant.
- If the specimen is broken or it is judged unsafe to remove from the specimen container, the LAU staff will discard the specimen container and its contents into an "autoclave and discard pan".

Note: If the Specimen Receiving Room staff detect contamination of the submittal form, their instructions are to put the original form into a plastic bag and then photocopy it. Both the original and the photocopy should be sent to Room B335 along with the specimen. If the photocopy is legible, the LAU supervisor or microbiologist will discard the original.

- The lab assistant will attach the specimen accession number to a piece of scotch tape on the outside of the plastic bag or to an empty tube if no specimen remaining.
- Proceed to check in specimen according to the directions listed III.A above.

LAB STAFF SERUM SAMPLES

- Initiate Lab-300 form if no form is received with the specimen.
- Take all specimens to the specimen receiving room for an accession number.

Disposition of Specimens other than Blood or Serum

Store tissues specimens to be held for future studies in the proper box (urine, rectal, other) in the -70⁰C freezer.in Room B337

Deliver non-frozen specimens to lab units or to freezer as instructed by LAU staff.

- Take slides for immunofluorescence (IF) to the isolation staff in Room B381 along with the extra numbered typed labels and a photocopy of the submittal form.

- CSF samples should be taken to Room B381 . If received frozen, store in the bottom shelf of the -70°C freezer; else store at 4°C on the lower right shelf in the refrigerator. In either case, place a photocopy of the submittal form and the extra labels in the slot designated for CSF samples.
- Stools for Norovirus (gastroenteritis) in the walk-in -20°C freezer in Room B351
- Store all other frozen samples in the -70°C freezer in Room B337.
- Store rectal swabs in the -70°C freezer in Room B337.
- Store paraffin embedded and samples received in formaldehyde in the box marked “hold for Shegio” in the 4°C refrigerator in B335.
- Store all other samples in the 4°C refrigerator in Room B337.

Processing Blood and Serum Specimens

Centrifuging Blood Specimens

- Blood specimens are centrifuged to separate the serum from the clot. Serum specimens are centrifuged to spin down fibrin clots or precipitates or red blood cells, etc.
- Carry specimens in wooden block (or wire rack).
- Place each specimen in a safety centrifuge cup ("sealed dome" cup).
- Balance centrifuge cups on the balance by adding distilled water to a balance tube if necessary.
- Snap safety cap on centrifuge cup.
- Blood (or bloody serum) samples should be spun at 2000 rpm for 15 minutes to separate the serum from the clot or pellet residual red blood cells.

CAUTION – When the centrifuge has stopped, carefully examine the centrifuge cups to be sure that no sample container has broken during centrifugation. If it appears that a sample has broken, leave the safety cap in place and consult a virologist from the SERO Section before proceeding further.

- Remove the specimen container from the centrifuge cup carefully so that the pelleted material is not disturbed.
- If the sample container is discovered to be broken when you are removing it, stop and consult a virologist from the SERO Section before proceeding further.
- As tube is removed from cup, be sure the label is still on the tube.
- Carry specimens in a block (or rack) to work area.

Transferring Serum to Storage Tubes

Note - Notify a virologist from the SERO Section if problems arise while processing or transferring specimens.

PREPARE STORAGE TUBES:

- Non-retrovirus samples are stored in 4 inch plastic, screw-capped tubes
- If volume is less than 0.3 ml, the sample is left in its original container and stored in the “short rack”. The 4 inch storage tube is left empty, labeled with appropriate accession number and placed in the regular storage rack with a note to “see short rack”.

- If "short" specimen has >0.3 ml, but less than 1.0 ml, transfer to a 1.5 ml Sarstedt tube. Place Sarstedt tube in the bottom of a 2 dram plastic vial which will allow it to be stored conveniently in the regular serum racks.
- If volume is greater or equal to 1 ml, prepare a second storage tube (1 ml plastic tube) for storage in the -700 C freezer in Room B350.
- Retrovirus samples are stored in 2 inch plastic, screw-capped tubes

PREPARE TRANSFER RACKS::

☛ It is recommended that empty spaces be left in the transfer rack so that samples can be moved either forward or to the left during the serum transfer process.

- Separate storage tubes by type [non-retro (including aliquot tubes) and retro]
- Label the storage tubes with the accession numbers corresponding to the samples to be transferred. The accession number label should be placed horizontally approximately ¼ in below the top of the 4" tubes and vertically on the retrovirus and aliquot tubes.

TRANSFER SAMPLES:

- Work in the Bio Safety Cabinet. Wear disposable gloves, change if they become contaminated.
- When removing any tube closure (i.e. snap-cap, vacutainer stopper, etc) except a screw-cap, place a small square of plastic bag paper over stoppers or caps. This procedure will reduce aerosols and glove contamination.
- Pick up the original specimen container and read the Accession Number and Patient ID
- Using transfer pipette or equivalent device, aseptically remove the specimen from the original tube taking care not to disturb the sediment or clot.
- Pick up and verify that the label on the storage tubes matches the sample from the original specimen container.
- Complete the sample transfer by delivering the specimen into the labelled storage tube(s)
- Place original retrovirus samples in the storage rack provided by the Retrovirus Unit.
- Place the unwanted stoppers, clots, vials, and tubes, from the original specimens in an autoclave pan with the "Autoclave, Discard" label with Room B335 written on it..

☛ Notes

- 1) Leave serum specimens in their original tubes if the volume is too small to transfer (see above).
- 2) Re-centrifuge specimens as described above if clot or sediment is disturbed while pipetting or if the serum sample still has residual red blood cells.
- 3) Leave survey specimens in their original tubes unless instructed to transfer by a virologist from the SERO Section.

Storage of Serum Samples

Filing Routine Serum Samples

- File sera in numerical order in the plastic serum rack in the 40C refrigerator in B335.
- Place an empty "place holder" tube with the sample accession label and explanation for the missing specimen (specimens to be returned from MDL, broken specimens, too short specimen, etc.) in the serum rack.

- File very short specimens in the block marked for "short specimens" and cross-file in the regular serum rack with an empty "place holder" tube file with accession number label and marked "short"
- File B, C, and etc. (duplicate) specimens in the rack on the top of the serology drawer file labeled "B" specimens.
- Label serology drawer files by the year and the specimen numbers using black or red pen alternating color by years.

SAMPLE:	2007
	101 - 400

Refiling Tested Serum Samples

- At the end of each day, refile in the serology file drawers those specimens that have been tested and returned to the rack marked "Refile".

Note - Refile sera returned to refrigerator by user. Tighten caps before refiling. Tubes should be cold when caps are tightened.

Filing Group Survey and Proficiency Testing (PT) Samples

- Samples for PT are stored in their original storage containers until testing is completed. They will then be stored (or discarded) by the VRDL Quality Control Officer
- The Section Supervisor responsible for the special study shall decide how study samples will be stored.

Preparation of Samples to be Referred to Another Laboratory

Samples will sometimes need to be referred to another laboratory (most frequently to CDC or MDL) for testing. The laboratory assistant(s) will be notified by e-mail by LAU staff or a Project Coordinator with specific instructions as to which laboratory and volume to be sent. Such notification should be sent at least one day prior to the specimen being referred if at all possible.

Note - The laboratory assistant will immediately notify the requestor and ask for instructions if she cannot comply with the referral request for any reason (i.e. sample is too short to make an aliquot of the requested volume and still retain a portion of the sample for VRDL). (Do not deplete specimen unless approved by Immunoserology or LAU virologist).

Note - In unusual circumstances the laboratory assistance will be directed to send the entire remaining volume of serum to another laboratory. In this case, the laboratory assistant will send the original tube to the designated laboratory and create an empty "place holder" tube with the sample accession number and explanation to file in the serum storage racks.

Samples Referred to CDC or another Reference Laboratory

PREPARING the ALIQUOT TUBE

- If the copies of the original accession number labels are no longer available, the laboratory assistant will request another set for Specimen Receiving staff.

Note - It is intended to attach a label printer to the computer in B335 so that labels can be printed by the laboratory assistant.

- Label a 1.5 ml Sarstedt aliquot with the VRDL accession number label.
- Wearing gloves and operating in the BSC, transfer the requested amount of serum into the aliquot tube.
- Tighten the screw cap and wrap tape around the cap in a “clock-wise” direction so that the cap is not inadvertently loosened while being taped.
- Place the aliquot in the plastic box in the refrigerator in B335 designated “Send to CDC”.

Samples Referred to MDL

- The laboratory assistant will follow the instructions for sending an aliquot to CDC except:
- The requestor will supply a StarLIMS “sample rejection report” which explain why the sample is being transferred.
- After tightening the screw-cap tube, the laboratory assistant will take the “sample rejection report” and the sample to the Specimen Receiving room so they can generate a MDL request form and assign it an MDL accession number.

Packaging and Shipping Instructions

✦ Typically samples are sent to laboratories within California using Golden State Overnight (GSO) as the courier. Samples sent to laboratories outside California are usually sent via FedEx.

Shipping Clinical Serum Samples

- Shipping paperwork (including the cover letter, any submittal forms, shipper’s waybill and appropriate package labels) will be prepared by office staff and sent to the laboratory assistant responsible for shipment.
- If a serum is to be sent, an e-mail as described above will be sent requesting that the sample be aliquoted.
- The laboratory assistant will remove the sample from the plastic box in the refrigerator in B335 designated “Send to CDC”.
- Wrap the sample in tissue paper and fasten with tape.
- Place one or more of the samples to be shipped in a Saf-T-Pak plastic shipping envelope and seal.
- Place the plastic envelope inside an outer Saf-T-Pak Tyvec shipping envelope and seal.
- Place the Tyvec envelope into an insulated shipping box
- Add a sufficient number of cold-packs (usually 3-4) to keep the sample at 40C during transport.
- Add packing material to fill the rest of the shipping container.
- Label the shipping container with the appropriate labels as shown below and take to the Mail Room for pickup.
- “Shipped By” label (including telephone number of person responsible for the sample)
- “Shipped To” label
- Shipper’s waybill
- “Category B” shipment label
- “Class 6.2” shipment label

- Return the info copy of the shipping list and photocopy of the waybill showing the tracking number to the requestor. These papers will be included in the patient's medical record to document shipment of the sample.

Shipping Clinical Tissue Samples

Note- Tissue samples are usually very small in volume and will subsequently thaw quickly. Putting dry ice in the insulated shipping box before retrieving the sample will keep the sample frozen while it is being packaged.

- Follow the same instructions as above except:
- Tissue samples are routinely shipped frozen on dry ice which requires the following changes:
- Place some dry ice inside the insulated shipping box before you retrieve the tissue sample from the appropriate Unit (usually the Isolation Unit).
- After placing Tyvac shipping envelope in the insulated box add additional dry ice to keep sample frozen during shipment. Usually 5-7 kilograms of dry ice is adequate.
- You must add a "Dry Ice" Label to the package and label it with the weight (in kilograms) of the dry ice
- File rest of the shipping box with packing material.
- If the sample is being sent by FedEx, you must check the box that states "Dangerous Goods – Shipper's Declaration not Required" and also check the box marked "Dry Ice".

Shipping Infectious Samples

Note - The packager must be currently certified to ship infectious samples.

- Follow the same instructions for shipping serum or tissue samples above except:
- The insulated shipping box must meet the specifications for Air Transport (Packing Instructions 650
- A "Shipper's Declaration" must be prepared with at least 2 copies
 - a) The original "Shipper's Declaration" plus one copy must be attached to the outside of the shipping box.
 - b) One copy which is retained by the appropriate VRDL MR Unit for 3 years.
- An additional "Infectious Substance" label must be included on the box.