VIRAL AND RICKETTSIAL DISEASE LABORATORY

GUIDELINES FOR LABORATORY SERVICES

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Version	Date	Author/Editor	Laboratory Director
6.0	7/01/2018	M. Salas	Dr. Carl Hanson, Acting

Brief Description of Changes:

1. **Updated VRDL homepage link**
2. **Updated section mission statements**
3. **Updated Local Public Health Laboratory (PHL) list to reflect closure of Placer County PHL**
4. **Added new supervisor contact for VPDHS**
5. **Added information about storage and shipment conditions for urine samples for mumps testing**
6. **Updated links to VRDL submittal form site**
7. **Updated links for information on the shipment of Biological Substances**
8. **Updated information on WNV testing by IgM EIA or PRNT**
9. **Added information on Yellow Fever testing at CDC**
10. **Updated CDPH Zika information link**
11. **Updated contacts for MERS consultants**
12. **Added availability of vaccine strain measles RT-PCR**
13. **Added availability of Hepatitis A virus testing**
14. **Added specimen types for arbovirus PRNT Appendix A**
15. **Added availability of SLE IgM EIA, Hepatitis A PCR, and rickettsia PCR to Appendix A**
16. **Updated HIV and HTLV test methods to Appendix A**
17. **Added Congenital Zika Syndrome to Appendix B**
18. **Table of Assays - Changed Flu B lineage genotyping from non-diagnostic to diagnostic**

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Introduction

This is an informational guide for clinical and public health laboratory staff regarding the availability of diagnostic laboratory services from the California Viral and Rickettsial Disease Laboratory (VRDL). However, it should be noted that service is subject to constant change as new services are offered and some diagnostic assays discontinued. The reader is strongly encouraged to visit the VRDL website at VRDL Website (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL.aspx). This site will be updated with the latest diagnostic assays and services, submittal forms and information on special projects

COMMONLY USED ABBREVIATIONS

term	*Organizations*	*term*	*definition*
CDPH	= California Department of Public Health	PCR	= polymerase chain reaction
VRDL	= CDPH Viral and Rickettsial Disease Laboratory	IF	= immunofluorescence assay (can be used for antibody (IFA) or antigen detection (DFA))
DCDC	= CDPH Division of Communicable Disease Control Branch	EIA	= enzyme immunoassay
MDL	= CDPH Microbial Disease Laboratory	WB	= Western blot
VBDB	= CDPH Vector Borne Diseases	Direct	= Direct antigen or nucleic acid detection
LCS	= CDPH Laboratory Central Services	RFFIT	= neutralizing test for rabies antibody
MELS	= Medical and Epidemiological Liaison Section (previously known as the Medical Records and Local Assistance Unit	IgG IgM	= immunoglobulin G = immunoglobulin M
HD	= Local City or County Health Department	HO	= local Health Officer
PHL	= Local County Public Health Laboratory	LD	= local PHL Director
LDT	Laboratory Developed Test		

History

The Viral and Rickettsial Disease Laboratory (VRDL) is the oldest state public health virology laboratory in the United States, established in 1939 as the Influenza Research Laboratory with support from the Rockefeller Foundation. Dr. Monroe Eaton was the first laboratory director. The VRDL began offering diagnostic services in 1943. In 1947 when leadership was passed to Dr. Edwin H. Lennette the laboratory could test for 14 viral agents or diseases. With a strong commitment to the development and evaluation of new viral assays, by 1976 the VRDL was able to perform tests to identify over 300 different viruses. *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections* edited by Dr. Lennette and VRDL team members is still widely used as a laboratory reference. Leadership passed to Dr. Richard Emmons in 1978; to Dr. Michael Ascher in 1994; to Dr. Mike Janda in 2001; to Dr. Carol Glaser in 2002; to Dr. David Schnurr in 2009; to Dr. Sharon Messenger in 2011; to Dr. Dongxiang Xia in 2012 and Dr. Carl Hanson who assumed the leadership in 2017 and is our current acting Laboratory Director. VRDL has been a highly recognized resource for laboratory diagnostic consultation, training and research with active collaboration with epidemiologists, clinicians and other partners.

Mission Statement

The Viral and Rickettsial Disease Laboratory provides laboratory support, technical assistance, and research required for the diagnosis, investigation, and control of viral and rickettsial diseases and for the development and maintenance of high quality local viral laboratory services in California. VRDL also provides consultation services to the staff of local public health laboratories, California Departments of Public Health (CDPH) and Health Care Services, and other state agencies. For counties not having available public health laboratory services, VRDL functions as the reference and local public health laboratory for viral and rickettsial diseases. As part of the Department's laboratory science training program, VRDL trains local public health laboratory personnel in state-of-the-art standardized laboratory procedures.

The VRDL is composed of five Sections that are responsible for the following functions:

The Vaccine Preventable Diseases and Herpesviruses Section (VPDHS) performs diagnostic testing for measles, mumps, rubella, varicella zoster (MMRV) and other herpesviruses, West Nile virus, and spotted fever group Rickettsia. We research and perform strain typing of MMRV and hepatitis A viruses to support public health investigations.

The Respiratory and Gastroenteric Diseases Section (RGDS) performs diagnostic testing to identify 21 types of viral respiratory agents including influenza, respiratory syncytial virus, rhinovirus and enterovirus as well as the bacterium Mycoplasma pneumoniae. The RGDS supports and coordinates the Respiratory Laboratory Network (RLN), a network of more than 26 PHLs throughout California whose mission is to provide testing for influenza at the local level and to respond to surge capacity events (e.g., emergence of a novel influenza or an upsurge in severe cases of respiratory

illness as seen with Enterovirus D68). This section serves as one of three National Influenza Surveillance Reference Centers in the United States. In this role, we collaborate with the US CDC to strain-type influenza viruses for situational awareness of influenza viruses circulating throughout California and detection of novel and potential pandemic influenza viruses. The RGDS also conducts antiviral resistance testing of selected influenza viruses to monitor for the emergence of drug resistant influenza viruses in California. The RGDS conducts testing of suspect acute viral gastroenteritis cases and are able to detect and identify norovirus (NV), sapovirus, astrovirus, rotavirus, and the gastroenteric adenoviruses. The RGDS supports and coordinates the Norovirus Laboratory Network, working in a similar fashion to the RLN, to provide local testing of suspect gastroenteritis outbreaks for more than 24 local PHLs. The RGDS is a CaliciNet-certified laboratory that performs strain-typing of noroviruses and is a member of CaliciNet, the CDC's national NV outbreak surveillance network. CaliciNet's goals are to catalogue and link NV outbreaks in the United States and monitor the emergence of novel NV strains.

The Zoonotic and Vectorborne Diseases Section (ZVBDS) is responsible for the identification of rabies virus and other animal and vector-borne diseases to support clinical diagnosis as well as disease surveillance. Methods for detection include virus isolation, fluorescent antibody and direct detection tests, molecular detection assays including strain typing of some viruses (rabies and Sin Nombre virus).

The Retroviral Diseases Section (RDS) serves as a statewide reference laboratory for HIV and other retroviruses and provides consultation to local PHL's and clinicians throughout the state. The section also provides neutralization testing for arboviruses including WNV, SLE, Dengue and Zika among others. Research activities include the development of new assays.

The Medical and Epidemiology Liaison Section (MELS) coordinates all diagnostic specimens received by VRDL for testing and answers questions regarding test availability, sample collection, and shipment and interpretation of test results. MELS clients include other branches of CDPH, local public health laboratories, clinical laboratories, and physicians throughout the state. This section coordinates several statewide surveillance efforts including the Neurologic Surveillance and Testing, West Nile Virus Surveillance and Sentinel Physician Influenza Surveillance in coordination with CDPH and Centers for Disease Control and Prevention (CDC).

Sources of Virology Services and Contact Information

To avoid costly duplication of services, the VRDL generally does not accept specimens for tests which are available locally. Samples are not accepted from private individuals. Individuals seeking virology testing must consult their private physician or go through their local health department. Specimens inadvertently submitted to the VRDL will be returned to the local public health laboratory. Physicians are urged to contact their local health department for information about the services that they can provide. If the requested tests are not performed locally, the local laboratory may:

- Receive and forward specimens to the VRDL
- Provide instructions, forms and containers for direct submission for services available by VRDL
- Refer the submitter to a clinical laboratory that can provide the test requested.

Local Public Health Laboratories

Note: For the most up-to-date contact information, visit the California Association of Public Health Laboratory Director's (CAPHLD) website at CAPHLD website (www.CAPHLD.org)

There are currently 30 approved local public health laboratories in California. Viral diagnostic services offered by these laboratories vary and are determined by their respective health officers. A few laboratories provide comprehensive viral diagnostic services. Most have some capability to perform viral serologic tests, virus isolation and antigen / nucleic acid direct detection. While not all laboratories are now equipped to perform all tests, services are continually being extended. (See table on following page.)

Local Public Health Laboratories and their contact information

Jurisdiction	Mailing Address	Phone	Fax
Alameda	2901 Peralta oaks Ct. 2nd Floor, Oakland, CA 94605	510-382-4300	510-382-4333
Butte	695 Oleander, Chico, CA 95926	530-891-2747	530-895-6660
Contra Costa	2500 Alhambra Ave. Rm 209, Martinez, CA 94553	925-370-5775	925-370-5252
Fresno	1221 Fulton Mall, Fresno, CA 93721	559-600-6370	559-660-7718
Humboldt	529 I Street, Eureka, CA 95501	707-268-2179	707-445-7640
Imperial	935 Broadway, El Centro, CA 92243	760-482-4437	760-353-9736
Kern	1800 Mt. Vernon Ave. 3rd Floor, Bakersfield, CA 93306	661-868-0505	661-868-0264
Kings	330 Campus Drive, Hanford, CA 93230	559-584-1401	559-583-8178
Long Beach City	2525 Grand Ave, Long Beach, CA 90815	562-570-4077	562-570-4070
Los Angeles	12750 Erickson Ave., Downey, CA 90242	562-658-1330	562-401-5995
Madera	14215 Road 28, Madera, CA 93638	559-675-7893	559-675-0478
Merced	260 East 15th Street, Merced, CA 95340	209-381-1297	209-381-1290
Monterey	1270 Natividad Road, Salinas, CA 93906	831-755-4636	831-757-4652
Napa / Solano / Yolo / Marin	2201 Courage Drive, Fairfield, CA 94533	707-784-4410	707-423-1979
Orange	1729 West 17th Street, Santa Ana, CA 92706	714-834-8385	714-834-7968
Riverside	4065 County Circle Drive, Riverside, CA 92503	951- 358-5070	951-358-5015
Sacramento	4600 Broadway, Suite 2300, Sacramento, CA 95820	916-874-9231	916-874-9432

Jurisdiction	Mailing Address	Phone	Fax
San Bernardino	150 E. Holt Blvd, Ontario, CA 91761, CA 92415	909-458-9430	909-986-3150
San Diego	3851 Rosecrans St, Suite 716, San Diego, CA 92186	619-692-8500	619-692-8558
San Francisco	101 Grove Street, Room 419, San Francisco, CA 94102	415-554-2800	415-431-0651
San Joaquin	1601 East Hazelton Ave., Stockton, CA 95205	209-468-3460	209-468-0639
San Luis Obispo	2191 Johnson Ave., San Luis Obispo, CA 93406	805-781-5507	805-781-1023
San Mateo	225 West 37th Ave. Rm 113, San Mateo, CA 94403	650-573-2500	650-573-2147
Santa Barbara	315 N. Camino Del Remedio Rm 262, , Santa Barbara, CA 93110	805-681-5255	805-681-4753
Santa Clara	2220 Moorpark Ave. 2nd Floor, San Jose, CA 95128	408-885-4272	408-885-4275
Santa Cruz	1080 Emeline Ave., Santa Cruz, CA 95060	831-454-5445	831-454-5000
Shasta	2650 Breslauer Way, Redding, CA 96001	530-225-5072	530-225-5061
Sonoma / Mendocino	3313 Chanate Road, Santa Rosa, CA 94504	707-565-4711	707-565-7839
Stanislaus	820 Scenic Dr., Modesto, CA 95350	209-558-7356	209-558-5343
Tulare	1062 S. K Street, Tulare, CA 93274	559-685-2684	559-713-2881
Ventura	2240 E. Gonzales Rd Suite 160, Oxnard, CA 93036	805-981-5131	805-981-5130

Viral and Rickettsial Disease Laboratory - Contact Guide

Please use the following table as a guide to decide who to call or e-mail for assistance.

CDPH Emergency Hotline: 1-888-273-4431

Dr. Carl Hanson, Acting Laboratory Director (510) 307-8540; Carl.Hanson@cdph.ca.gov		
VRDL phone (510) 307-8585; fax (510) 307-8599		
Section / Special Surveillance	Section Chief / Surveillance Director	Section Supervisors / Project Coordinators
Zoonotic & Vectorborne Diseases Section	Sharon Messenger, PhD (510) 307-8623 Sharon.Messenger@cdph.ca.gov	Kristina Hsieh, DrPH (510) 412-1501 Kristina.Hsieh@cdph.ca.gov
Respiratory & Gastroenteric Diseases Section; Influenza Sentinel Providers; Unexplained Respiratory Death Surveillance	Debra Wadford, PhD, MS, PHM (510) 307-8624 Debra.Wadford@cdph.ca.gov	Hugo Guevara (510) 307-8565 Hugo.Guevara@cdph.ca.gov
Vaccine Preventable Diseases and Herpesviruses Section	Jill Hacker, PhD (510) 307-8538 Jill.Hacker@cdph.ca.gov	Rick Berumen (510) 231-4185 Ricardo.Berumen@cdph.ca.gov
Retroviral Diseases Section	Carl Hanson, PhD (510) 307-8540 Carl.Hanson@cdph.ca.gov	Peter Patiris (510) 377-6887 Peter.Patiris@cdph.ca.gov
Medical & Epidemiology Liaison Section (510) 307-8585		Peter Patiris (510) 377-6887 Peter.Patiris@cdph.ca.gov
Neurological Surveillance Testing		Meghana Madala (510) 307-8562 Meghana.Madala@cdph.ca.gov
Arbovirus Surveillance		Maria L. Salas, MPH (510) 307-8606 Maria.Salas@cdph.ca.gov

Other Contact Information at the California Department of Public Health

Cantact	Telephone Number
Microbiology Disease Laboratory (MDL)	(510) 412-3700
Infectious Disease Branch (IDB)	(510) 620-3434
Veterinary Public Health Section (IDB VPHS)	(916) 552-9740
Vector Borne Disease Section (IDB_VBDS)	(916) 552-9730
Immunization Branch (IZB)	(510) 620-3737
Communicable Disease Emergency Response Branch (CDER)	(510) 231-6861

Types of Service Provided

The VRDL offers various levels of service depending on the type of submitter. The VRDL:

- provides routine diagnostic laboratory services for certain counties.
- is the reference laboratory for all private clinical and public health laboratories in the state. (**Note**: Private clinical laboratories should be referred to their local public health laboratory if they are located in a health jurisdiction that has one.)
- accepts specimens for the purpose of referring them to the Centers for Disease Control and Prevention (CDC). This testing is primarily for agents that are not endemic in California and for which we do not have specific reagents.
- may accept specimens from non-California submitters with the approval of the Laboratory Director or Medical Officer.

Specimen Collection, Storage and Shipment Guidelines

Note: In order to ensure accurate patient and specimen identification, submitter must provide the following information:

Patient Name or Patient Identification Number (must also be written on the sample container)
Date of Birth
Date of Onset (estimate if necessary since this is very important to result interpretation)
Type of Specimen(s) (must also be written on the sample container)
Date Specimen collected (must also be written on the sample container)

SPECIMENS	WHEN TO COLLECT	PREFERRED AMOUNT (IF YOU HAVE LESS – CALL THE VRDL FOR A CONSULTATION)	REQUIRED COLLECTION MEDIUM	STORAGE AND SHIPMENT CONDITIONS	
				Delivery to VRDL within 72 hrs	Delivery to VRDL greater than 72 hrs
Blood or serum for antibody or molecular assays (*See note #1*) Plasma is acceptable for HIV and HTLV assays	Acute phase- ASAP (no later than 7 days). Convalescent phase- 14-28 days after onset	2.5 – 5ml of clotted blood or 1-2.5 ml of serum	None	2°- 8°C / none or cold pack	2°- 8°C / none or cold pack
Respiratory Samples Nasopharyngeal, throat & nasal swab, endotracheal aspirates, bronchial washing *See note #2*	ASAP – not later than 5 days after onset	1 – 2 swabs –if 2 swabs put into a single VTM vial	2-3 ml of viral transport medium (VTM) Note#7	2°- 8°C / cold pack	-70^{0}C / Dry Ice
Buccal swabs for suspected mumps *See note #2*	ASAP – not later than 9 days after onset	1 – 2 swabs –if 2 swabs put into a single VTM vial	2-3 ml of viral transport medium (VTM) Note#7	2°- 8°C / cold pack	-70^{0}C / Dry Ice
Stool for direct detection (Polymerase Chain Reaction) *See note #3*	ASAP – not later than 7 days after onset	2-4 grams	None	2°- 8°C / cold pack	2°- 8°C / cold pack
Rectal swabs for isolation or direct detection (PCR) *See note #3*	ASAP – not later than 7 days after onset	1 – 2 swabs	2-3 ml of viral transport medium	2°- 8°C / cold pack	-70^{0}C / Dry Ice
Cerebrospinal fluid (CSF) *See note #4*	ASAP – not later than 3 days	1 – 3 ml	None	2°- 8°C / cold pack	-70^{0}C / Dry Ice
Biopsy tissue		As much as available	Sufficient to keep sample moist	2°- 8°C / cold pack	-70^{0}C / Dry Ice
Autopsy tissue	ASAP – within 24 hrs of death	½" - 1" cube of each sample	None	2°- 8°C / cold pack	-70^{0}C / Dry Ice
Vesicular lesion fluid, basal cells from skin lesions; - for PCR	ASAP – before crusting stage	1 -2 swabs	2-3 ml of viral transport medium (VTM)	2°- 8°C / cold pack	-70^{0}C / Dry Ice
Smears from skin lesions	ASAP – before crusting stage	1 – 2 slides each with 3 cell spots	Air dry no fixation	None	None
Eschar swab for rickettsial testing	ASAP	1 swab per eschar	Send dry	2°- 8°C / cold pack	2°- 8°C / cold pack
Urine *See note #5*	ASAP - within 7 days	10 – 40 ml	*See note #6*	*See note #6*	*See note #6*
For any sample not described above – please call the VRDL at (510) 307-8585 for a consultation					

Table Notes:

1. Acute blood specimens (taken ASAP but within 7 days of the date of onset)- acute specimens of particularly high public health significance for which reliable

molecular or IgM antibody assays exist are tested as soon as possible. Testing a single acute blood for other requests is generally not useful and a convalescent specimen (collected 14-28 days after onset) should be requested to determine if the patient is responding to an infection by increasing antibody production (the "gold standard" to associate an agent with the patient's current illness).

2. Types of swabs, collection sites and holding media to be used for viral samples:

DACRON swabs with plastic shaft should be used to collect samples for virus isolation and PCR..
A buccal swab is the specimen of choice for mumps isolation attempts and PCR testing.
To preserve the infectivity of NP, nose and/or throat swabs use 2-3 ml of VTM to protect the swab.
Bacterial transport media such as LQ Stuart (green or red top), Amies (with or without charcoal) and A.C.T.I. contain antiviral substances and render the sample UNSATISFACTORY for virus isolation or PCR.
Cotton or cotton alginate swabs and wooden handles contain oils which are inhibitory to viral growth and render the sample UNSATISFACTORY for virus isolation or PCR attempts.
Stools are superior to rectal swabs for virus isolation and/or PCR. VRDL reserves the option of not testing rectal swabs.
A minimum of 4 and maximum of 10 stools should be submitted from gasteroenteritis outbreaks where norovirus is the suspected agent. Stool is superior to rectal swabs for virus isolation and/or PCR. VRDL reserves the option of not testing rectal swabs.
Rectal swabs are a poor substitute for stool samples and should only be sent if stool samples absolutely cannot be obtained. Use 1-2 ml of Viral Transport Medium (VTM) to protect the swab. VRDL reserves the option of not testing rectal swabs.

3. CSF specimens can be tested for antibody, PCR, or or virus isolation depending on a number of factors such as agent suspected, onset date relative to collection date and availability of test.

A CSF specimen (1) taken within a few days of the date of onset and shipped promptly at 2°- 8°C via an overnight delivery service or (2) promptly frozen and shipped frozen, are usually of more value for virus isolation or PCR assay. This is especially true when there is a corresponding blood specimen that can be tested for antibodies. CSF specimens which are contaminated with blood are not satisfactory for antibody testing.
A CSF specimen which does not meet the criteria above but has a corresponding blood specimen is held pending the outcome of the serology results on the blood sample. If antibody is detected in the serum, the CSF sample may be tested if CSF is validated as an acceptable sample for the agent requested.

4. Storage and shipment conditions for urine samples that cannot be delivered to the VRDL within 48 hours of collection are specific to the suspected agent.

Urine specimens are no longer considered to be the “Specimen of Choice” for suspected cases of mumps or rubella. We recommend throat or NP swabs for rubella, and buccal or oropharyngeal swabs for mumps.
Urine can continue to be submitted for detection of measles or mumps by PCR, although a buccal swab should be collected concurrently for mumps testing. Urine should be spun down and the cell pellet resuspended in 2 -3 ml of VTM if the sample must be frozen.

VRDL Specimen Submittal Forms

The VRDL General Purpose Specimen Submittal Form Lab 300 is a barcoded, fillable PDF form designed to improve accuracy and speed of specimen accession This form should be used for most submissions. The current version of the form will always be found at VRDL Submittal Form (https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/VRDL_General_Purpose_Specimen_Submittal_Form.pdf) and should be completed electronically, not handwritten, and a hard copy should accompany the specimen. One form should be completed for each specimen submitted.

In addition, VRDL has several supplementary submittal forms customized to provide specific specimen collection instructions and obtain additional clinical and epidemiological information specific to the patient’s illness. The most up-to-date versions of these forms are available in PDF format on the VRDL website at Submittal Form Page (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL_Specimen_Submittal_Forms.aspx). Some examples include:

- Gastroenteritis Outbreak Information Summary Form
- Hantavirus Submittal Instructions, Screening Criteria, and Case Report Form
- Influenza Reference Examination Submittal Form
- Rabies Virus – Specimen Submittal Form for Suspected Rabid Animals

Shipment of Clinical Samples

Note: per Dr. Kathleen Haines (CDC Division of Select Agents and Toxins, phone (404) 718-2102 or email wtj9@cdc.gov):

- FedEx is no longer accepting Select Agents for transport
- The only vendor willing to ship select agents and toxins at the current time is World Courier (1-800-221-6600, Option 1)

Currently clinical samples are divided into three categories: Unregulated, Biological Substance - Category B, and Biological Substance - Category A. The definitions for these three categories can be found in the IATA Dangerous Goods Regulations (IATA 1.0) and the Code of Federal Regulations (49CFR 171.8). Rules and regulations for the shipment of clinical diagnostic samples and infectious agents are subject to change and more stringent rules can be established by any individual carrier. Currently the rules for shipping samples by air (regulated by IATA/ICAO) are the most stringent. The following

guidelines are provided for your convenience. You should check with your carrier for any changes or more stringent requirements. It is the responsibility of the organization presenting the package to the carrier to determine the correct method of preparing and packaging the sample for shipment. It should be assumed that the package will go by air unless you know it will be delivered by ground transport.

Unregulated

Samples classified as Unregulated are known to not contain any agent capable of infecting humans or animals.

Biological Substance – Category B (UN 3373)

Defined by exclusion as any clinical sample that does not meet the definition of Biological Substance – Category A. In general Category B is applicable for all clinical samples that are being shipped for diagnostic purposes including virus isolates being shipped for further characterization (such as influenza virus for strain typing). Patient samples are considered to be Category B and are defined as collected directly from humans or animals, including but not limited to excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Biological Substance – Category A (UN 2814)

Category A substance or agent is one that is capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals. Category A substances have more stringent packaging rules which includes:

- Packager must be currently certified as an Infectious Substance Shipper.
- Shipper's Dangerous Goods Declaration must accompany the package.
- 24/7 contact phone number must be provided in case the package leaks during transportation.

Possible Select Agent

Follow the guidelines above for a Category A substance when shipping a clinical sample with a high likelihood of containing a Select Agent to a reference laboratory for testing. The general USDA permit is required, but not a select agent permit. **Note**: A CDC/USDA form 2 does not have to be completed unless you are transferring a confirmed select agent.

Further information on the shipment of Biological Substances is available at the following websites:

Name	Link
United States Department of Transportation (DOT)	*DOT: (https://www.phmsa.dot.gov/transporting-infectious-substances/transporting-infectious-substances-safely)*

Name	Link
International Air Transport Association (IATA)	IATA: (https://www.iata.org/publications/store/Pages/infectious-substances-shipping-guidelines.aspx)

Testing Samples Outside of Normal Business Hours

VRDL business hours are Monday – Friday from 8:00 AM to 5:00 PM. The VRDL is closed for all State and Federal holidays. Requests for testing outside of normal business hours require prior consultation and approval.
If testing is approved, the following actions will help ensure that samples are delivered to the laboratory.

For weekend testing, the submitter must either hand-deliver the sample or use a transportation service that will guarantee Saturday delivery. The submitter must mark the waybill for "Saturday delivery" and specify "ring bell at gate for admittance" under Special Instructions.

The courier Golden State Overnight has an early Saturday morning delivery by 8:00 AM.

Upon prior consultation and approval, FedEx samples can be addressed to the FedEx Station address 1600 63rd Street Emeryville, CA 94608 and marked as "hold for pick up." These samples can be picked up at 9:00 AM by one of the VRDL staff.

If a transportation company is used, the submitter must fax a copy of the waybill (showing the shipper's tracking number) to the VRDL during normal business hours.

Requests for Laboratory Results

Results may only be reported to the original submitting laboratory or health department. Results may also be reported to the local health jurisdiction of the submitter and/or patient. Requests by other parties should be referred to the original submitter who can provide them a copy of the results. Results will normally be sent by secure email to the submitter. The person requesting a fax must guarantee that the fax machine is in a secure, non-public location.

In cases of high public health importance or where time is of the essence, laboratory results are reported verbally, by fax, and/or secure email. If the laboratory results are still pending and a reasonable time has elapsed, please call the VRDL at (510) 307-8585. VRDL staff will investigate the reason the results are delayed, provide an estimated time the results will be available and determine if a preliminary report can be issued.

Prior Consultation for Unusually Hazardous Samples

Please call the VRDL if you believe that you are sending the VRDL samples that may contain unusually hazardous agents. The VRDL Director (or designee) will consult as to the best way of shipping these samples and will make special arrangements for receiving and handling of these samples. Examples of unusually hazardous samples include suspected cases of avian (or other pandemic) influenza or Ebola and possible

BT agents (such as “white powder”). Note that special shipping rules and regulations apply to shipping Select Agents.

Arboviruses

The VRDL has serologic and molecular assays for select arthopod-borne viruses. West Nile virus (WNV) is the arthropod-borne virus (arbovirus) most commonly identified in California patients. Several other arboviruses, including St. Louis encephalitis (SLE), Western equine encephalitis (WEE), and California serogroup viruses (California encephalitis (CEV) and Jamestown Canyon (JCV) viruses), although rare, have been documented in California. Arboviruses of importance that are not endemic to California include dengue, Yellow Fever, Japanese encephalitis (JE), chikungunya (CHIK) and Zika viruses.

Clinically important arbovirus families include the Flaviviridae (e.g., WNV, Dengue, Zika, SLE, or JE), Togaviridae (genus Alphavirus, e.g., WEE and CHIK), and Bunyaviridae (e.g., CEV and JCV). Arboviral infections commonly are identified using serology, but due to the high degree of serological cross-reactivity between arboviruses within a family, exact diagnosis can be challenging. For example, a single serum can be reactive to both WNV and SLE and thus appear IgM or IgG positive for both viruses. Similarly, WEE and CHIK are serologically cross-reactive, as are the California serogroup viruses. IgM antibody typically is more specific than IgG, but IgM to these viruses may persist for months to years and thus confound the interpretation. PCR can be a powerful tool available for arboviral diagnosis, but due to a transient viremia, specimens must be collected early in the disease course.

Specimen collection and shipment to VRDL

Collection of serum (and CSF in neuroinvasive cases in which an LP is performed) for detection of virus-specific antibody is recommended for laboratory confirmation of arboviral disease. For some arboviruses (*e.g.*, dengue, WNV, CHIK), molecular detection or virus isolation can be performed on acute samples of serum and CSF.

Serum:

Collect 5-7 ml of blood in a red top or serum separator tube (SST).
The optimal time for collection of acute blood for molecular or serological assays is as soon as arboviral disease is suspected and up to 7 days after symptom onset.
If initial testing is negative and an arbovirus is strongly suspected, it may be worth submitting another ‘acute’ serum for testing (just a few days after first one obtained).
A convalescent serum sample should be collected 10-30 days after symptom onset.
Paired acute and convalescent sera are a useful epidemiologic tool for confirmation of an acute infection. Paired serum specimens taken early (i.e., acute) and at least 2-3 weeks later (i.e., convalescent) can be tested by indirect immunofluorescent (IFA) or by

plaque reduction neutralization test (PRNT) to test for a four-fold or greater increase in antibody titer to different arboviruses.
The specimens should be spun and the serum removed from the clot.

CSF: Collect 2-3 ml of CSF in a sterile collection tube.

Samples should be transported on cold packs as soon as possible following collection. If samples cannot be transported immediately, they can be held at 4°C for 72 hours before shipping. Otherwise, specimens should be frozen, preferably at -70°C, and shipped on dry ice.

Flaviviruses

Data on prior Flavivirus immunizations (*e.g.*, for yellow fever or Japanese encephalitis viruses) and travel history are important for the proper interpretation of serological test results for any Flavivirus (*e.g.*, WNV, Dengue, SLE). A plaque reduction neutralization (PRNT) assay may help resolve indeterminate results but is only available for epidemiologic purposes.

West Nile Virus (WNV) – Requests for WNV testing at VRDL are coordinated by the WNV human surveillance program. Please consult with VRDL for guidance any time WNV is strongly suspected, regardless of previous test results. WNV testing may include:

- Repeat testing of serum IgM will be performed only when requested.
- Testing for IgG will only be performed on paired acute and convalescent sera.
- CSF samples will be tested for WNV IgM by EIA and, if collected early, RT-PCR;
- Acute sera (collected within 7 days of onset) may be tested for WNV RNA by RT-PCR for epidemiologic purposes;
- Immunofluorescence assay (IFA) may be done as an adjunct test on serum only;
- Non-diagnostic plaque reduction neutralization testing (PRNT) will be perfomed on WNV IgM-positive specimens; WNV IgM results from local public health or commercial laboratories should be included on the submittal form.

Notes:

If the IgM is negative in the serum sample but you strongly suspect WNV, another serum sample should be collected 2-3 days after the first serum. WNV IgM is usually present in immunocompetent individuals by day 5 of illness onset.
In immunocompromised individuals, the WNV antibody response may be delayed. For these patients, additional testing is warranted. Please consult with VRDL for guidance.
Enterovirus PCR may also be done on CSF specimens on a seasonal basis. Call 510-307-8585 to find out whether the most current algorithm includes enterovirus PCR.

Dengue Virus (types 1-4) – Competent mosquito vectors for dengue virus have been identified in some California counties, although none have tested positive for dengue. However, testing will be considered for residents or cases with travel to these areas or with travel history to an endemic area (e.g., Mexico, Caribbean, Tahiti, Southeast Asia and India, etc.).

Dengue testing may include:

- EIA and IFA for IgM and IgG antibodies. Serologic assays do not distinguish between types; testing for IgG will only be performed on paired acute and convalescent sera.
- Real-time RT-PCR for acute serum specimens. This test will distinguish between dengue types (Dengue 1-4). Blood for PCR should be collected within 10 days of symptom onset.

St. Louis encephalitis (SLE) – Historically a significant cause of arboviral encephalitis in California, SLE remerged in California in 2015 after an 18 year absence. SLE tests currently are offered for surveillance only. Information on current SLE and WNV activity in California and testing recommendations can be found at CA WNV Website: (http://www.westnile.ca.gov/).

Japanese encephalitis (JE) virus – JE virus is endemic to Asia and the Western Pacific. Serum from persons vaccinated against JE virus will cross-react in other flavivirus serological tests.

- Requests for JE virus testing will be sent to CDC.

Yellow Fever (YF) virus – YF virus is endemic to tropical and sub-tropical Africa and South America. Serum from persons vaccinated for YF will cross-react in other flavivirus serological tests.

- Requests for YF virus testing will be sent to CDC; specific clinical and exposure history is required prior to submitting specimens for testing. Please contact the VRDL Medical and Epidemiology Liaison Section at 510-307-8585 for information on the preapproval process for YF testing at CDC.

Zika Virus – Originally isolated in Uganda's Zika Forest in 1947, Zika virus is now endemic in Southeast Asia, the South Pacific and most recently in Central and South America, and Mexico. Zika Virus will cross-react with other flavivirus serological tests. For up to date testing information please visit the CDPH Zika web site at: CDPH Zika website: (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Zika.aspx)

Alphaviruses

Western equine encephalitis (WEE) – There have been 639 cases of WEE identified in the US since 1964 (CDC data). The most recent case of WEE in California occurred in 1986.

- WEE neutralizing antibodies are assayed by WEE/Chik PRNT as a differential titration.

Chikungunya (CHIK) – CHIK is not endemic in California; however, it is increasingly diagnosed in travellers returning from chikungunya-endemic areas. Patient should have a travel history to an endemic area (e.g., Mexico, Caribbean, Oceania, Southeast Asia and India, etc.).

- Serum samples are tested for CHIK IgM by EIA or IFA and CHIK IgG by IFA. Reactive specimens are confirmed by PRNT.
- Real-time RT-PCR for acute serum or plasma specimens. This test has been validated for diagnostic testing. Blood for PCR should be collected within 10 days of symptom onset.
- Samples may also be forwarded to CDC for additional testing by serology, PCR and virus isolation.

Bunyaviruses

California Encephalitis Virus (CEV) and **JCV**: These viruses are endemic to California, but testing is not offered as a routine service since these viruses rarely are known to cause disease here.

- Requests for CEV and JCV will be sent to CDC for testing.

Ebola Virus

- No sample may be submitted for testing unless prior approval has been obtained. Health Care Providers must contact their local public health department for assistance with assessing the patient.

- Ebola virus is a Risk Group 4 agent which requires special handling and shipping. Your local public health department can provide shipping and handling information.

- Ebola testing is performed by PCR on whole blood preserved with EDTA, clot activator, sodium polanethol sulfonate (SPS) or citrate in plastic collection tubes.

- Do NOT submit specimens in glass containers or preserved with Heparin (Green Top) tubes.

Neurologic Surveillance and Testing (NST)

Initiated in March 2012, Neurologic Surveillance and Testing (NST) is designed to provide enhanced diagnostic testing and consultation for cases of unexplained neurologic illnesses that are presumed to be of infectious origin. Cases that will be considered for testing include those who meet the following criteria:

Clinical Characteristics	Rapidly progressive encephalitis, paralysis with associated encephalitis, neurologic illness with associated rash, OR unexplained culture negative meningitis that appears to be bacterial

	.
Exposures	Epi-links to other case(s) of neurologic illness; foreign travel ≤3 weeks; animal bite/scratch (especially wildlife); thought to be related to a recent immunization (≤4 weeks); significant mosquito or tick exposure (e.g. outdoor activities in non-urban areas)
Unexplained Neurological Fatalities	Hallmarks of infection (e.g. fever, rash, CSF pleocytosis); in particular, pediatric deaths

Testing will be tailored to each case as determined by clinical picture, laboratory values, and exposure history. **Note**: anti-NMDA receptor testing is NOT performed at VRDL.

To send samples to NST for testing, ALL of the following requirements must be met:

1. Physicians must obtain approval **PRIOR TO** the submission of patient samples.This can be accomplished by filling out the NST case history form and sending it via fax or email to 916-440-5940 or NeuroSurveillance@cdph.ca.gov. You will be notified if your case is appropriate to send.
2. A completed 2-page Case History Form and Specimen Submittal Form must be sent along with a **full set** of specimens. **Required** samples are:
   - □ CSF (2-3cc)
   - □ Acute Serum (2-3cc in red or tiger top tube)
   - □ NP/Throat swab (in viral transport)

   Additional samples may be requested, such as a rectal swab, stool, or convalescent serum. Tissues will be requested for autopsy cases, and additional samples may be requested for suspect rabies cases.

   *****Note that samples will not be tested unless all requirements are met*****
3. The local public health department must be notified of all cases submitted for testing. **Note: Regardless of our involvement, encephalitis and meningitis are reportable conditions in California as per Title 17, Section 2500.**

Once a case has been approved and the above requirements met, samples can be shipped via an overnight courier such as FedEx or GSO to the address below:

ATTN: Specimen Receiving
Neurologic Surveillance and Testing
Viral and Rickettsial Disease Laboratory
850 Marina Bay Parkway
Richmond, CA 94804

Packages should be sent for delivery Monday through Friday only. For questions, please call Srimati Datta at (510) 620-3747

Creutzfeldt-Jakob Disease (CJD)

14-3-3 and Tau tests on CSF samples are available at the National Prion Disease Laboratory at Case Western Reserve. Autopsy provides the only method to firmly diagnose CJD. Samples may be sent via a commercial clinical laboratory. Contact the National Prion Disease Laboratory at (216) 368-0587 or visit Prion Diease Lab: (http://www.cjdsurveillance.com) for submittal forms and to arrange testing.

Gastroenteritis Samples

Note: Outbreaks are reportable under the Title 17, California Code of Regulations. Please communicate with your local communicable disease control unit to ensure that any norovirus oubreaks are reported to the California Department of Public Health - Statistics and Surveillance Section.

Norovirus PCR testing is intended for use primarily as laboratory support for epidemiological investigations. Specific case history, group submittal and instruction forms are available on the VRDL website or can be faxed upon request.

- Desired Specimen Type – Fresh stool collected undiluted in a sealed specimen container. Note that vomitus may contain lower norovirus titers, our PCR assay was validated to test stool samples.
- Timing - Ideally stool specimens should be obtained as soon as possible (within the first 48-72 hours of onset of diarrhea). This is the acute phase of illness while the stools are still liquid or semisolid and the amount of virus being excreted is greatest. The increased sensitivity of molecular assays (PCR) often allows the virus to be detected in stools collected up to 7-10 days after onset. For specimens collected late in the illness, the utility of viral diagnosis and interpretation of the test results is unclear and should be discussed with laboratory personnel before tests are conducted.
- Number of Samples – CDC requires a minimum of two (2) positive samples for norovirus before they will consider norovirus to be the causative agent for the outbreak. Thus, for meaningful laboratory results (see interpretation below) specimens from a minimum of four (4) to a maximum of ten (10) ill persons should be obtained during the acute phase of illness. The greater the number of stool samples submitted, the more meaningful the test results. A single stool sample with either positive or negative result will not be meaningful in an outbreak investigation. Additionally, testing of asymptomatic cases is not encouraged and will not be tested without prior consultation.
- Storage and Transportation - Stool specimens should be kept refrigerated at 2°-8°C until they can be sent to the laboratory. Samples stored at this temperature can be kept for 2-3 weeks without compromising diagnostic yield. Samples can be frozen if samples cannot be delivered to the laboratory promptly.

VRDL follows the CDC interpretative guidelines to evaluate laboratory PCR results:

- Positive - Norovirus can be considered to be the etiologic agent if norovirus nucleic acid is detected in two (2) or more stools per outbreak.

- Negative - To be considered negative for norovirus, at least four (4) or more acute stool samples (all collected with 7-10 days of onset of diarrhea) must be submitted and all must be negative for norovirus nucleic acid.

Norovirus strain typing (PCR positives from the Local HD)

Local health departments are strongly encouraged to submit two or more positive RNA-extract and/or stool samples from each outbreak attributed to Norovirus in their health jurisdiction. Such samples will be included in the norovirus strain typing project to determine which strains of norovirus are circulating in California. A norovirus RNA submittal form is available on the VRDL website or can be faxed upon request.

Hantavirus Pulmonary Syndrome (HPS)

The Sin Nombre Virus (causative agent of HPS) is endemic in California however the incidence of human infection is rare. Please obtain the CDC HPS Case Definition and Case History Form from the VRDL website or call VRDL to receive a copy by fax. Antibody testing for IgG and IgM is the gold standard test for this disease. However, since the incidence of HPS is low in California, we strongly recommend that you also submit a respiratory specimen (nasopharyngeal swabs or washes, tracheal aspirates, bronchoalveolar lavage and/or pleural fluid) for viral isolation and/or respiratory PCR assays to test for other agents that may be causing your patient's illness.

- Specimen Submittal Instruction- Fill out the HPS case history form as completely as possible. Fax one copy to (510) 307-8578 and send a copy with the blood specimen.
- Collect two tubes of whole blood (one 5ml tube in EDTA; one 10 ml whole clotted blood. Send samples on a "cold pack" to the VRDL laboratory at the address shown below using an overnight delivery service.
- Collect an NP swab and/or lower respiratory sample (such as an ET aspirate or bronchial wash).
- It is very important to use an overnight delivery service because the EDTA samples will begin to degrade within three days.

In addition, request your laboratory to save all specimens (including hematology differential slides) from the patient until HPS serology has been completed. If the patient is deceased, call the laboratory for shipping instructions for paraffin embedded lung and kidney, and/or fresh frozen lung and kidney (these latter tissues should be held frozen at -70°C). In cases where our HPS results are equivocal or inconsistent with the clinical presentation, specimens may be forwarded for further testing to the Centers for Disease Control and Prevention.

Immunity Status Requests

Requests for immunity testing is not a routine service except in cases of high public health significance such as:
Measles case contacts, when requested for epidemiological investigation support.
Varicella case contacts. It is the responsibility of the employer to determine the immune status of their health care workers. Upon prior consultation, the VRDL may agree to test health care workers who were exposed to a varicella case and are uncertain of their immune status.
Rabies immunity for staff of public health laboratories responsible for testing rabies samples. This may be extended to limited numbers of other health department employees including veterinarians under contract to open animal heads.
Note: Rabies immunity status is determined by the Rabies Rapid Fluorescent Foci Inhibition Test (RFFIT) which measures neutralizing antibody. This test is labor intensive and is currently only performed once every three (3) months. Due to the limited numbers of samples that can be tested, prior approval is required for all non-public health laboratory staff.
Note: Due to technical difficulties, the RFFIT antibody assay is currently not being offered by VRDL. Until this test becomes available again, we recommend sending rabies immunity samples to the Rabies Laboratory at Kansas State University which performs testing on a weekly basis at a very reasonable cost. Information and submittal forms can be obtained from their website at Kansas State University Lab: (http://www.ksvdl.org/rabies-laboratory/rffit-test/index.html) or by phone at (785) 532-4483.

Rabies

The VRDL serves as the statewide reference laboratory for rabies. VRDL performs rabies testing for counties lacking a full-service public health laboratory, as well as supports local public health laboratories by providing confirmatory testing. VRDL performs rabies virus variant-typing on specimens in which rabies virus is detected by the direct fluorescent antibody assay (rabies DFA) and maintains an archive of characterized rabies virus variants circulating in California.

Rabies (Human)

For cases of suspected human rabies, call the VRDL Medical Epidemiology and Liaison Section (MELS) at (510) 307-8585 for a consultation.

Rabies (Animal)

Animal rabies case definition
Rabies testing is restricted to species known to be susceptible to infection with rabies virus (i.e., species within class Mammalia). Efforts should be made to limit testing to

animals that have behavioral history (such as unprovoked bite) and clinical signs compatible with and supportive of rabies encephalopathy.

Requests for animal testing should be referred to your local health department. Local health departments may request animal rabies testing by VRDL if there has been significant human exposure or for confirmatory testing.

Brain tissue received by VRDL will be tested for the presence of rabies virus antigen using current VRDL procedures which closely follow the "Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing: A Minimum Standard for Rabies Diagnosis in the United States" (Rabies Standards: (http://www.cdc.gov/rabies/pdf/RabiesDFASPv2.pdf)).

Weekend and Holiday Testing Policy - VRDL normal business hours are Monday – Friday from 8:00 AM to 5:00 PM. The VRDL is closed for all State and Federal holidays. Requests for testing outside of normal business hours require prior consultation and must be approved by the VRDL.

Specimen collection

- Whenever possible, the brain of the animal should be removed. The entire head may be sent for bats or other small animals.
- Ideal specimens to submit for rabies testing are, in order of preference:
    - Whole brain
    - Complete cross-sections of brain stem and bilateral samples of cerebellum.
        - Bilateral samples of hippocampus may be submitted in addition to or instead of cerebellum (if cerebellum is not available).
    - For large animals, in addition to the complete cross-sections of brain stem and bilateral samples of cerebellum, submissions of complete cross sections of both hippocampi and representative bilateral segments of the cerebral cortex are strongly encouraged.

Specimen storage

- Do NOT formalin-fix the brain tissue.
- Fresh brain material should be kept cold (2-4^{o}C) during storage and transit.
    - If delivery to VRDL cannot be ensured within 48 hours of collection, freezing (-20^{o}C) the tissue may be advised, but repeated freeze-thaw cycles can negatively affect the sensitivity of the test.
- Brain tissue segments should be placed in separate petri dishes or other clean/sterile container.
    - clearly label each container to indicate which brain segment is present, the species type, date of collection, and unique identifier if appropriate.
    - parafilm-wrap each container to avoid leakage.

Specimen submission

- Complete the VRDL Rabies Specimen Submission form with as much information as is available.
- Specimens transported to VRDL must follow Department of Transportation (DOT) and/or International Air Transport Association (IATA) shipping guidelines to ensure proper packaging and biocontainment of the specimens during transport.
- Specimens may be shipped to VRDL at the following address:

California Department of Public Health
ATTN: Specimen Receiving
850 Marina Bay Parkway
Richmond, CA 94804
Phone: (510) 307-8585

Communications

- Upon completion of testing--usually the same day the sample is received--VRDL will:
  - report preliminary results via telephone
  - prepare a written laboratory report with the and deliver within 24-48 hours of test completion

Respiratory Viruses

MERS

Specimen submission

MERS testing will not be performed unless prior approval is obtained from the following designated MERS consultants at 510-620-3737:

Dr. Robert Schechter Robert.Schechter@cdph.ca.gov
Christina Moore Christina.Moore@cdph.ca.gov

VRDL follows the MERS EUA protocol and CDC recommendations for MERS rRT-PCR Assay:

- Lower respiratory tract specimen (most important) such as a sputum, tracheal aspirate or BAL
- NP/OP swab – we can obtain the NP swab and OP swab separately and then place the swabs together in a single vial of UTM or VTM and test as a single specimen,
- Serum
- Note: Routine testing for stool in a MERS PUI is no longer recommended.

If, instead of serum, we receive plasma or whole blood then we will test, but these specimen types will be reported out as unsatisfactory (if MERS negative) and explain that serum is the recommended specimen type.

Retroviruses

HIV Serology

- All specimens sent to our laboratory for HIV testing are screened by 4th generation Enzyme Immunoassay (EIA) (Bio-Rad HIV-1 p24 antigen, HIV1/2 antibody combo). EIA non-reactive specimens are reported as "HIV-1 p24 antigen, HIV-1/2 antibody not detected". Repeat reactive specimens are tested by Lateral Flow Immunochromatographic Assay (Bio-Rad Geenius HIV1/2 Supplemental Assay). Results and additional recommendations are reported in accordance with CDC/APHL guidelines for HIV testing.
- Dried blood spots are no longer accepted for HIV serology.

HTLV Serology

- Specimens are screened by EIA (Avioq Inc.) and IFA (LDT).
- Antibody positive specimensare typed by IFA endpoint titration
- If EIA and IFA results are discrepant or the IFA is inconclusive (reactive on one antigen and not the other) or unsatisfactory (nonspecific), sample is reflexed for Western blot (LDT).

Rickettsial Agents

Specimen Collection for Rickettsia, Anaplasma, and Ehrlichia Testing

Note: Treatment decisions should be based on epidemiologic and clinical evidence and should never be delayed while awaiting confirmation by laboratory results.

Multiple rickettsial diseases are endemic in California, including Rocky Mountain Spotted Fever (*Rickettsia rickettsii*), murine typhus (*R. typhi* and *R. felis*), human granulocytic anaplasmosis (*Anaplasma phagocytophilum*), human monocytic ehrlichiosis (*Ehrlichia chaffeensis*), and the newly identified "Pacific Coast tick fever" (*R. philipii* or *Rickettsia* 364D).

Serology

The indirect immunofluorescence assay (IFA) is generally considered the reference standard for rickettsial infections and is used by VRDL to test for *R. rickettsii*, *R. typhi, Anaplasma phagocytophilum,* and *Ehrlichia chaffeensis*.

- Cross-reactivity among rickettsial group antigens is common. Thus, a high degree of serologic cross-reactivity may occur between the various Spotted Fever group rickettsia (SFGR) such as *R. rickettsii* and *R. philipii,* as well as between the typhus group rickettsia, *R. typhi* and *R. felis.*

- **Paired acute and convalescent sera are important** for confirmation of an acute infection. Paired serum specimens taken early (*i.e.,* acute) and at least 2-3 weeks later (*i.e.*, convalescent) are preferred in order to test for a four-fold or greater increase in antibody titer. Patients may lack diagnostic IgG and IgM antibody titers in the first 7 days of illness, but most patients demonstrate increased IgM and/or IgG titers by the second week of illness. Other factors, such as persistent antibody titers in some individuals for years after an exposure to some *Rickettsiae* and a late IgG response up to 4 weeks after illness onset in some individuals, highlight the need for paired sera.

Minimum specimen requirement:

- Acute **AND** convalescent sera (5-10 cc) should be collected in a red top or tiger top tube, to allow for optimal testing.

Molecular Testing

Direct Detection of Spotted Fever Group Rickettsia by PCR. This test allows detection of the spotted fever group of *Rickettsia*, including *R. rickettsii* and the newly identified human pathogen *R. philipii* (formerly known as 364D strain), which has been identified in multiple counties in California.

Minimum specimen requirement: For all cases where ANY rickettsial infection is suspected, the following samples should be requested for PCR:

- Eschar/scab (if present) **OR**
- Swab of eschar, open lesions, pustules or vesicles

    1. Eschar/scabs and swabs should be sent in dry, sterile containers. Do not add saline or other transport medium. Ship to VRDL using an overnight courier or within 48 hours.

    2. If no eschar or other lesion is present and a rickettsial infection is suspected, collect an acute phase whole blood (5-10 cc) in an EDTA purple top tube.

    3. Punch biopsies of rash (if an eschar is not present) are no longer recommended. If obtained, punch biopsy specimens should be stored at 2°- 8°C in a sterile gauze pad slightly dampened with sterile saline and shipped to VRDL (preferably overnight or within 48 hours).

- Acute phase whole blood (5-10 cc) collected in an EDTA purple top tube

- Additional desired specimens, as available:
  The VRDL is developing new real-time PCR tests for diagnosis of rickettsial infections, including *R. rickettsii, R. felis*, and *R. typhi*. To assist with public health

surveillance in all cases where ANY rickettsial infection is suspected, acute phase whole blood may be tested for non-diagnostic, epidemiologic purposes or may be forwarded to CDC for additional testing.

Vaccine Preventable Diseases

Measles

See Measles Testing Information for more detailed specimen and testing guidance https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/Measles-Testing-InformationVRDL.pdf

The VRDL performs real-time reverse transcription-PCR, serology, and molecular genotyping for measles. All laboratory results should be interpreted in conjunction with relevant epidemiologic and clinical history for ruling in or ruling out an acute measles infection.

Note that in recently vaccinated persons (6-45 days prior to rash onset), neither IgM nor IgG responses can distinguish measles disease from a vaccination response. Measles PCR and genotyping can be used to distinguish between a vaccine and wild-type strains.

We encourage submission of samples for both serological and direct detection (PCR) whenever possible.

PCR (preferred method) – Measles PCR is a very sensitive and specific test; however, a negative result cannot rule out measles, particularly if the specimen is of poor quality or taken too late after illness onset. A new vaccine strain measles RT-PCR assay is now available. Suspected measles respiratory samples that are negative for measles RNA may be reflexed for respiratory virus molecular testing.

- **PCR specimen requirements –** Specimens should be collected as soon as measles is suspected.
    - ***A respiratory swab*** collected up to 9 days after rash onset. A throat swab is preferred followed by nasopharyngeal or nasal swab. All respiratory swabs submitted for measles should be shipped in 2-3 ml of VTM or UTM; do not use Amies, liquid Stuart, or other bacterial media.
        - **Note:** Flocked swabs are preferred for specimen collection.
        - **Note:** Nasal aspirates should be centrifuged at 2500 x g for 15 minutes at 2°- 8°C and the pellet resuspended in 1 ml of VTM, then stored and shipped at -70°C or colder. If these conditions are not available, then the entire sample should be stored and shipped at 2°- 8°C by overnight delivery service.

- ***Urine*** collected up to 10 days after rash onset. Collect up to 10-50 ml of urine, collecting from the first part of the urine stream if possible. Process by centrifuging at 500-600 *x g* for 5-10 minutes at 4C. Resuspend the pellet in 2-3 ml of VTM. Store and ship at -70°C or colder. If these conditions are not available, then the entire urine sample should be stored and shipped at 2°-8°C by overnight delivery service.

Samples which can be received by the VRDL within 48 hours should be stored and shipped at 2° - 8°C. Otherwise swabs and processed urine should be stored and shipped at -70°C or colder.

Genotyping – All measles PCR-positive specimens will be genotyped to help inform public health interventions. VRDL requests that any positive sample identified in a local public health laboratory be forwarded to VRDL for typing. *Please provide your laboratory results, including Ct value, when forwarding samples for typing.*

Serology – Enzyme immunoassay (EIA) and immunofluorescent assay (IFA) are available for both measles IgM and IgG antibodies.

- Minimum specimen requirement
  - Draw 2-5 cc in a red top tube.
  - Collect an acute serum concurrently with respiratory or urine specimens.
  - Collect a convalescent serum 2-4 weeks after symptom onset.
  - The specimens should be spun and the serum removed from the clot.

Note: In cases where collection of specimens may be difficult (e.g., infants), VRDL can test serum collected in capillary tubes, although this is not optimal. To obtain adequate sample volume, approximately 3 capillary tubes of blood should be collected. Capillary tubes should be capped and placed in another larger tube for protection before transport.

- **IgM** – IgM results should be interpreted in conjunction with measles PCR results.
  - Measles IgM can be detected in an unvaccinated person in approximately 70% of acute samples taken at least 3 days after rash onset; confidence increases to 99% in a sample taken 7 days after rash onset.
  - Previously vaccinated persons may not demonstrate an IgM response.
  - Other diseases, such as parvovirus infection, infectious mononucleosis, or rheumatologic disease can cause false positive measles IgM results.

- **IgG** – Obtain both acute and convalescent serum samples to confirm a measles diagnosis by 4-fold rise or greater in IgG titer. The acute sample can be tested for both IgM and IgG. Collect a convalescent sample 2-4 weeks after symptom onset for paired IgG testing.
  - A single positive measles IgG result cannot distinguish between a recent or past infection or vaccination, or the presence of maternal antibody (in infants <15 months).

- Paired acute and convalescent serum specimens that demonstrate a 4-fold rise or greater in IgG titer or seroconversion from IgG negative to positive are considered confirmatory for a recent measles infection.

Mumps

See Mumps Laboratory Information for more detailed specimen and testing guidance VRDL Mumps Guidance: (https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/Mumps_Testing_VRDL.pdf). The VRDL performs real-time reverse transcription-PCR, serology, and molecular genotyping for mumps. All laboratory results should be interpreted in conjunction with relevant epidemiologic and clinical history for ruling in or ruling out an acute mumps infection.

PCR (preferred method) – Mumps PCR is a very sensitive and specific test; however, a negative result cannot rule out mumps, particularly if the specimen is of poor quality or taken too late after illness onset. Suspected mumps respiratory samples that are negative for mumps virus RNA may be reflexed for respiratory virus molecular testing.

- **PCR specimen requirements –** Specimens should be collected as soon as mumps is suspected.
  - **Buccal** (ideal) or other oral (e.g., throat) swabs collected up to 9 days after onset of parotitis are accepted. Place swabs in 2-3 ml of VTM. Samples which can be received by the VRDL within 72 hours should be stored and shipped at 2°- 8°C. Otherwise swabs should be stored and shipped at -70°C or colder.
  - For proper buccal swab collection see Mumps Laboratory Information VRDL Mumps Guidance: (https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/Mumps_Testing_VRDL.pdf)
  - Urine has poorer diagnostic sensitivity, but can be tested if collected up to 10 days after parotitis onset. Collect up to 10-50 ml of urine, collecting from the first part of the urine stream if possible. Process by centrifuging at 500-600 x g for 5-10 minutes at 4C. Resuspend the pellet in 2-3 ml of VTM. Store and ship at -70°C or colder. If these conditions are not available, then the entire urine sample should be stored and shipped at 2°- 8°C by overnight delivery service.

Genotyping – All mumps PCR-positive specimens will be genotyped to help inform public health interventions. VRDL requests that any positive sample identified in a local public health laboratory be forwarded to VRDL for typing. *Please provide your laboratory results, including Ct value, when forwarding samples for typing.*

Serology – The VRDL can test for both IgM and IgG to mumps.

- Minimum specimen requirement
  - Collect 7-10 cc in a red top tube concurrently with respiratory specimens.
  - Collect a convalescent serum 2-4 weeks after symptom onset.

- The specimens should be spun and the serum removed from the clot.

- Interpretation
  - In vaccinated persons, mumps IgM results may be falsely negative, and paired serum specimens may not show a rise in IgG titer.
  - False positive IgM or non-specific IgM reactions are known to occur in other diseases, such as parainfluenza virus, Epstein-Barr virus, and human herpesvirus 6.

Rubella

The VRDL performs real-time reverse transcription-PCR, serology, and molecular genotyping for rubella. Results should always be interpreted in conjunction with the relevant clinical and epidemiologic risk factors. Rubella and measles should both be included in the differential diagnosis of patients presenting with an acute generalized rash and fever.

PCR (preferred method) – Rubella PCR is a very sensitive and specific test; however, a negative result cannot rule out rubella, particularly if the specimen is of poor quality or taken too late after illness onset.

- **PCR and culture specimen requirements –** Specimens should be collected as soon as rubella is suspected.
  - **A respiratory specimen** collected within 7-10 days of symptom onset (nasopharyngeal, nasal or throat swabs or washes). All respiratory swabs submitted for rubella should be shipped in 2-3 ml of VTM or UTM; do not use Amies or other bacterial media.
  - Urine is not currently an accepted specimen for rubella PCR but may be useful in cases of congenital rubella syndrome. Collect up to 10-50 ml from the first part of the urine stream if possible. Process by centrifuging at 500-600 x g for 5-10 minutes at 4C. Resuspend the pellet in 2-3 ml of VTM. Store and ship at -70°C or colder. If these conditions are not available, then the entire urine sample should be stored and shipped at 2°- 8°C by overnight delivery service.
  - Most specimens submitted for rubella PCR will also be tested for measles.

Genotyping – Specimens positive by PCR will be genotyped for epidemiological purposes.

Serology – The VRDL performs an EIA assay to measure rubella IgG and IgM antibody. It should be noted that there is potential non-specific cross-reaction with parvovirus IgM.

- Minimum specimen requirement
  - Collect 7-10 cc in a red top tube concurrently with respiratory specimens.
  - Collect a convalescent serum 2-4 weeks after symptom onset.
  - The specimens should be spun and the serum removed from the clot.

Hepatitis A virus

Molecular testing and strain typing - Molecular testing and strain typing is available for surveillance purposes only. With approval from your local health jurisdiction, please submit at least 0.5 ml of frozen, HAV IgM-positive serum. The VRDL does not offer HAV IgM testing.

Polio

Real-time reverse transcription PCR and sequence analysis - Poliovirus is an enterovirus (EV) and can be ruled out at VRDL by following this algorithm: initial testing for enterovirus by real-time reverse transcription-PCR (rRT-PCR) assay followed by molecular typing of EV positive samples by Sanger sequence analysis to identify the EV type. Please note there are hundreds of EV types, but sequence analysis can distinguish Poliovirus from other EVs.

The VRDL does not offer poliovirus immunity status testing.

Polio Vaccine Strain - Samples from patients suspected of being infected with the vaccine strain of polio will be forwarded to CDC for strain typing.

Varicella – Chickenpox and Herpes Zoster (Shingles)

PCR and Direct Detection – The optimal tests for cases of suspected chickenpox or shingles are either PCR of scabs or dry swabs from an unroofed lesion or direct fluorescence assay (DFA) of lesion smears. The VRDL performs a PCR assay that can distinguish between wild-type and vaccine strains of VZV.

- Minimum specimen requirement
  - **For PCR,** the ideal specimens include scabs and dry lesion swabs. In cases with neurological symptoms, cerebrospinal fluid can also be tested.
    - Remove several scabs (a glass slide is useful for this purpose) and place in a clean, dry container.
    - Swab basal cells from the unroofed lesion. Place swab in clean, dry container.
    - Swabs submitted for PCR should be sent dry rather than diluted in VTM
  - **For DFA,** prepare a slide smear. Remove the scab of the lesion, discarding any pus and then collect cells from the base of the lesion using a **Dacron** swab with plastic handle. Use the swab to make a smear on a microscope slide.
    - Note: The smear must contain at least 30 cells to be a valid sample. It may be necessary to collect basal cells from several lesions to obtain the required minimum number of cells.
    - The same swab can be placed in a dry container for PCR or into 1-2 ml of VTM (for isolation attempts).

Genotyping – Specimens positive by PCR will be genotyped for epidemiological purposes.

Serology – An enzyme immunoassay (EIA) is available to detect IgM and IgG to VZV.

- **Note:** A positive EIA IgG result in a single serum specimen will not indicate whether that person is protected from future exposure to VZV, nor can it distinguish between a recent or prior infection or immunization.

Prions

See Creutzfeldt-Jakob disease (CJD)

Referring Samples to Local HD, MDL and/or CDC

VRDL staff may refer samples to other laboratories upon review. Samples are routinely referred to another laboratory if:

- The sample was mistakenly addressed to the VRDL and the test is performed by MDL
- The VRDL does not provide routine diagnostic service to that county
- The county provides the requested test
- The test requested is only performed at the CDC

Appendix A Table of VRDL Assays – Sorted by Agent - Updated: 7/1/2018

The table below shows VRDL assays that are currently validated per CLIA requirements and are routinely available, unless otherwise specified,. This table is subject to frequent change as new assays are developed and validated.

Note: a status of "non-diagnostic" means that the assay is performed for surveillance purposes or when authorized for special circumstances.

Unless otherwise specified:

- Whole clotted blood may be substituted for serum
- Respiratory includes NP, nose & throat, throat, bronchial washes or ET aspirates
- Sputum is unsatisfactory for PCR

TAT is given in calendar days. Testing for all urgent requests and/or public health emergencies will be expedited and may displace our normal TAT for routine samples. Prior approval is required when requesting expedited testing.

Exceptions: See page 16 for TAT for samples submitted for Neurological Surveillance and Testing (NST). For requests for Arbovirus testing during the "off-season", add 7 days to the indicated TAT

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Arboviruses						
Chikungunya	IgG & IgM	IFA	Diagnostic	Travel history required	14 days	Serum
Chikungunya	IgM	EIA	Diagnostic		14 days	Serum
Chikungunya	Neutralizing Antibody	PRNT	Non-diagnostic		28 days	Serum/CSF
Chikungunya	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Serum / Plasma, CSF

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Dengue (does not distinguish type)	IgG & IgM	EIA	Diagnostic	Travel history required	30 days	Serum
Dengue (does not distinguish type)	IgG & IgM	IFA	Diagnostic		14 days	Serum
Dengue (does not distinguish type)	Neutralizing Antibody	PRNT	Non-diagnostic		28 days	Serum/CSF
Dengue (does distinguish type)	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Serum, CSF
St. Louis Encephalitis (SLE)	IgM	EIA	Non-diagnostic	Case history required	28 days	Serum, CSF
St. Louis Encephalitis (SLE)	Neutralizing Antibody	PRNT	Non-diagnostic		28 days	Serum/CSF
West Nile Virus (WNV)	IgG & IgM	EIA	Diagnostic	Case history required	14 days	Serum
West Nile Virus (WNV)	IgG & IgM	IFA	Diagnostic		14 days	Serum
West Nile Virus (WNV)	IgM	EIA	Diagnostic		14 days	CSF
West Nile Virus (WNV)	Neutralizing Antibody	PRNT	Diagnostic		28 days	Serum/CSF

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
West Nile Virus (WNV)	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	CSF
West Nile Virus (WNV)	Nucleic acid	real-time RT-PCR	Non-diagnostic		28 days	Serum
Western Equine Encephalitis (WEE)	Neutralizing Antibody	PRNT	Non-diagnostic	Case history required	28 days	Serum/CSF
Zika Virus	Nucleic Acid	real-time RT-PCR	Diagnostic	Travel history required	14 days	Serum, Urine, CSF, Amniotic Fluid
Zika Virus	IgM	IFA	Diagnostic		14 days	Serum
Zika Virus	IgM	EIA	Diagnostic		14 days	Serum
Zika Virus	Neutralizing Antibody	PRNT	Diagnostic		28 days	Serum

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Other Viruses and Rickettsial Agents in alphabetical order.						
Adenovirus	Isolation	Cell culture	Non-diagnostic		60 days	Respiratory
Adenovirus	Nucleic acid	real-time PCR	Diagnostic		14 days	Respiratory
Adenovirus	Typing	Sequence Analysis	Non-diagnostic	Prior consulation required	30-60 days	Respiratory, CSF or stool
Balamuthia Acanthamoeba and Nagleria	IgG	IFA	Non-diagnostic			Serum
Balamuthia Acanthamoeba and Nagleria	Nucleic acid	real-time PCR	Non-diagnostic	Refrigerated		CSF
Balamuthia Acanthamoeba and Nagleria	Isolation	Cell culture	Non-diagnostic	Non-frozen		Fresh brain
Coxiella burnetii (Q fever)	Phase II IgG	IFA	Diagnostic		14 days	Serum
Coxiella burnetii (Q fever)	Phase I IgG	IFA	Diagnostic		14 days	Serum
Coxiella burnetii (Q fever)		IFA	Diagnostic		14 days	Serum
Coxiella burnetii (Q fever)	Nucleic acid	real-time PCR	Non-routine (See LRN, below)	Prior consultation required	2 days	Blood

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Ehrlichia chaffeensis (HME)	IgG	IFA	Diagnostic	Case history required	14 days	Serum
Anaplasma phagocytophilum (HGA)	IgG	IFA	Diagnostic		14 days	Serum
Enterovirus	Nucleic acid	real-time RT- PCR	Diagnostic		14 days	Respiratory or CSF
Enterovirus	Isolation	Cell Culture	Non-diagnostic		60 days	Respiratory or Fecal
Enterovirus D-68 specific	Nucleic acid	real-time RT- PCR	Diagnostic		14 days	Respiratory
Enterovirus	Typing	Sequence Analysis	Non-diagnostic	Prior consulation required	30-60 days	Respiratory or CSF
Epstein-Barr Virus (EBV)	Nucleic acid	real-time PCR	Diagnostic		21 days	CSF
Hepatitis A Virus	Nucleic acid	Sequence Analysis	Non-diagnostic	Prior consulation required	28 days	Serum, plasma
Herpes simplex virus (HSV) (does not distinguish type)	IgG & IgM	EIA	Diagnostic		14 days	Serum

Viral and/or Rickettsial Agents	Analyte	Method	Status	Special Requirements	TAT (See note above)	Sample
Herpes simplex virus (HSV) (does not distinguish type)	IgG	EIA	Diagnostic	Requires corresponding serum	14 days	CSF
Herpes simplex virus - type 1	Nucleic acid	real-time PCR	Diagnostic		21 days	CSF or Lesion swab
Herpes simplex virus - type 2	Nucleic acid	real-time PCR	Diagnostic		21 days	CSF or Lesion swab
Herpes simplex virus (HSV)	Isolation	Cell Culture	Non-diagnostic		60 days	Oral swab or Cells from base of lesion
Human Coronavirus 229E	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Human Coronavirus HKU1	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Human Coronavirus NL63	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Human Coronavirus OC43	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Human Herpes Virus 6 (HHV6)	Nucleic acid	real-time PCR	Diagnostic		21 days	CSF
Human metapneumnovirus (hMPV)	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Human Immunodeficiency Virus (HIV-Combo Ag/Ab)	IgG / IgM P24 antigen	EIA	Diagnostic		14 days	Serum or plasma
Human Immunodeficiency Virus 1/2 (HIV-1/2)	IgG	Geenius	Diagnostic		7 days	Serum or plasma
Human T Cell Lymphotrophic Virus (HTLV) I & II	IgG	EIA	Diagnostic		14 days	Serum or plasma
Human T Cell Lymphotrophic Virus (HTLV) I & II	IgG	IFA	Diagnostic		14 days	Serum or plasma
Influenza A	Isolation	Cell Culture	Non-diagnostic		60 days	Respiratory
Influenza A	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory

Viral and/or Rickettsial Agents	Analyte	Method	Status	Special Requirements	TAT (See note above)	Sample
Influenza A subtyping for H1, H3, H5 and Pandemic Influenza A (H1) 2009, (H7)	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Influenza A B screening	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Influenza B	Isolation	Cell Culture	Non-diagnostic		60 days	Respiratory
Influenza B	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Influenza B Lineage	Genotyping	Real-time RT-PCR	Diagnostic		30-60 days	Respiratory
Isolate for Identification	Isolation	Cell Culture	Non-diagnostic		60-120 days	Cell culture isolate
Lymphocytic choriomeningitis (LCM)	IgG	IFA	Diagnostic		14 days	Serum
Measles (rubeola)	IgG & IgM	EIA	Diagnostic	For suspected cases, please contact the local public health department and	7 days	Serum
Measles (rubeola)	IgG	EIA	Diagnostic		7 days	CSF
Measles (rubeola)	IgG & IgM	IFA	Diagnostic		7 days	Serum

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Measles (rubeola)	Nucleic acid	real-time RT-PCR	Diagnostic	complete a case history form or enter into CalREDIE For CSF IgG antibody testing requires corresponding serum	7 days	Respiratory and Urine
Measles (rubeola)	Genotyping	Sequence Analysis	Non-diagnostic		28 days	Respiratory, urine or RNA extracts
Orf, Cowpox	IgG & IgM	IFA	Diagnostic		14 days	Serum
Orf, Cowpox	Antigen	DFA	Non-diagnostic		5 days	Vesicular swab / scab
Mumps	IgG	EIA	Diagnostic	For suspected cases, please contact the local public health department and complete a case history form or enter into CalREDIE	14 days	Serum
Mumps	IgG & IgM	IFA	Diagnostic		14 days	Serum
Mumps	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Buccal swab
Mumps	Genotyping	Sequence Analysis	Non-diagnostic		28 days	Respiratory or RNA extracts
Mycoplasma pneumoniae	Nucleic acid	real-time PCR	Diagnostic		28 days	Respiratory or CSF
Norovirus (includes Norwalk virus) (Winter Vomiting Disease)	Nucleic acid	real-time RT-PCR	Non-diagnostic	Stools from outbreaks only	14 days	4-10 Stools per outbreak
Norovirus strain typing	Nucleic acid	RT-PCR then Sequence Analysis	Non-diagnostic	Nucleic acid extracts from outbreaks	120 days	RNA positive extracts

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Parainfluenza types 1 - 4	Isolation	Cell Culture	Non-diagnostic		60 days	Respiratory
Parainfluenza types 1 - 4	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Rabies (suspected human case)	IgG and IgM	IFA	Diagnostic	Suspected Human Rabies requires case history and prior consultation with MELS (510) 307-8585	3 days	Serum
Rabies (suspected human case)	Antigen	DFA	Diagnostic		3 days	Various - Call for medical consultation
Rabies (immune status) ***Temporarily Unavailable***	IgG	RFFIT	Diagnostic	Limited to PH staff ***Temporarily Unavailable***	120 days	Serum
Rabies (animal)	Antigen	DFA	Diagnostic		3 days	Cross section of brain stem and cerebellum
Respiratory syncytial (RSV)	Nucleic acid	real-time RT -PCR	Diagnostic		14 days	Respiratory
Rhinovirus	Isolation	Cell Culture	Non-diagnostic		60 days	Respiratory
Rhinovirus	Nucleic acid	real-time RT-PCR	Diagnostic		14 days	Respiratory
Rhinovirus	Typing	Sequence Analysis	Non-diagnostic	Prior consulation required	30-60 days	Respiratory or CSF

Viral and/or Rickettsial Agents	Analyte	Method	Status	Special Requirements	TAT (See note above)	Sample
Rickettsia, Spotted Fever Group (SFG)	Nucleic acid	real-time PCR	Diagnostic	Case history requested	14 days	Eschar and lesion swab
Rickettsia typhi (typhus)	IgG	IFA	Diagnostic		14 days	Serum
Rocky Mountain spotted fever (RMSF)	IgG	IFA	Diagnostic		14 days	Serum
Rocky Mountain spotted fever (RMSF)	Nucleic acid	real-time PCR	Non-diagnostic		14 days	EDTA Whole Blood/serum/plasma
R. felis/R. typhi	Nucleic acid	real-time PCR	Non-diagnostic		14 days	EDTA Whole Blood/serum/plasma
Rubella (German measles)	IgG & IgM	EIA	Diagnostic	For suspected cases, please contact the local public health department and complete a case history form or enter into CalREDIE	14 days	Serum
Rubella (German measles)	Nucleic acid	real-time RT- PCR	Diagnostic		14 days	Respiratory
Rubella (German measles)	Genotyping	Sequence Analysis	Non-diagnostic		28 days	Respiratory or RNA extracts
Sin Nombre Virus Hantavirus Pulmonary Syndrome	IgG & IgM	EIA	Diagnostic	Case history required Prior consultation required	3 days	Serum

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
Vaccinia (vaccine strain)	IgG	IFA	Diagnostic	Prior consultation required	14 days	Serum
Varicella-zoster (Chickenpox & Shingles)	IgG & IgM	EIA	Diagnostic		14 days	Serum
Varicella-zoster (Chickenpox & Shingles)	IgG	EIA	Diagnostic	Requires corresponding serum	14 days	CSF
Varicella-zoster (Chickenpox & Shingles)	Antigen	DFA	Diagnostic		3 days	Lesion swab and/or Basal Cells from lesion
Varicella-zoster (Chickenpox & Shingles)	Nucleic acid	real-time PCR	Diagnostic		14 days	Scab, Lesion swab and/or Basal Cells from lesion/ CSF
Varicella-zoster (Chickenpox & Shingles)	Genotyping	Sequence Analysis	Non-diagnostic		28 days	Scab, Lesion swab and/or basal cells from lesion or DNA extracts
The use of LRN PCR assays are restricted to the investigation of possible BT events or other Public Health emergencies						
Ebola	Nucleic acid	real-time PCR	Non-routine	**Prior consultation required**	1 day	EDTA Whole blood

VIRAL AND/OR RICKETTSIAL AGENTS	ANALYTE	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT (SEE NOTE ABOVE)	SAMPLE
MERS-CoV	Nucleic acid	Real-time PCR	Non-routine	**Prior consultation required**	7 days	Respiratory
Non-Variola Orthopox Note #1 ***Temporarily Unavailable***	Nucleic acid	real-time PCR	Non-routine	**Prior consultation required** ***Temporarily Unavailable***	2 days	Scabs / Lesion swab
Q fever	Nucleic acid	real-time PCR	Non-routine	**Prior consultation required**	2 days	blood
Vaccinia (vaccine strain)	Nucleic acid	real-time PCR	Non-routine	**Prior consultation required**	2 days	Scabs / Lesion swab
Varicella	Nucleic acid	real-time PCR	Non-routine	**Prior consultation required**	2 days	Scabs / Lesion swab
Note #1 Acceptable sample types for the LRN Non Variola Orthopox PCR assay are: dried vesicular fluid on a slide (touch prep), fresh biopsy, skin or crust from roof of vesicle, dry or wet swab of lesion, cellular material from tissue culture demonstrating cytopathic effect. ***However, this assay is temporarily unavailable. Samples will be forwarded to the CDC for testing.***						

Appendix B Table of Viral and Rickettsial Diseases and their Causative Agents

Tables arranged by Disease or Syndrome and the likely etiologic agent is listed first followed by other agents in descending order of likelihood.

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Section # 1 - NERVOUS SYSTEM		
Aseptic Meningitis	Fever, headache, stiff neck. Spinal fluid leucocytes >10-500; rarely 1000 or more, predominantly lymphocytes. No paralysis or abnormal neurological findings.	Enteroviruses (coxsackie, echo, polio) Arboviruses (WEE, SLE) Mumps virus Herpesviruses LCMV various others, rarely adenovirus
Encephalitis Meningoencephalitis	Similar to aseptic meningitis plus one or more typical encephalitic signs such as marked drowsiness, stupor, confusion, dizziness, tremors, restlessness, seizures, abnormal reflexes.	Arboviruses (WEE, SLE, CEV) Enteroviruses Herpesviruses Post-infectious mumps, measles, rubella, influenza
Rabies	Acute encephalitis. Early clinical signs and symptoms of rabies, including headache, fever, chills, cough or sore throat, anorexia, nausea, vomiting and malaise, are vague and commonly confused with other conditions. Disease progresses rapidly (within 1-2 weeks) after clinical onset to include other symptoms more reminiscent of rabies, such as altered mental status (e.g., hyperactivity and agitation), paresthesia at the site of the animal bite, aero- and/or hydrophobia, hypersalivation due to muscle spasms and difficulty swallowing, and ultimately autonomic instability.	Rabies virus

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Poliomyelitis, Myelitis, Meningomyelitis	Similar to aseptic meningitis plus; muscle pain, weakness of one or more muscle groups with absent or diminished reflexes; often bladder weakness with urinary retention. No loss of sensory function.	Poliovirus (types 1, 2, 3) rarely other enteroviruses
Radiculo-neuritis, Guillain-Barre Peripheral neuritis	Typically sensory changes or loss; paresthesia, tingling, etc.; weakness or paralysis (typically symmetrical). CSF shows high protein (100 mg%); low leukocytes (10-15).	No known specific agent; probably secondary to various acute infections (enteroviruses and/or respiratory viruses)
Section #2 - RESPIRATORY INFECTIONS		
Upper respiratory disease (URI), common cold	Coryza; with or without sore throat, hoarseness, slight cough, slight or no fever.	Rhinovirus, coronavirus; adenovirus, influenza, parainfluenza, respiratory syncytial (RSV)
Croup; laryngotracheitis	Coryza; fever; hoarseness; deep, persistent cough. Most common in children up to age 6 or 7.	Parainfluenza (types 1, 2); other parainfluenza, occasionally adenovirus, influenza, RSV
Bronchiolitis	Coryza; fever; cough; wheezing; labored expiration. Neonates and infants through 3 or 4 years of age.	RSV (esp. neonates-3 months), parainfluenza type 3 (infants-5yrs); occasionally parainfluenza, others
Influenza	Fever; muscle aches; marked malaise; deep cough. Coryza usually follows. Pneumonia in severe cases. Rarely myocarditis, encephalopathy, Reye Syndrome.	Influenza A, Influenza B

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Viral pneumonia, Atypical pneumonia, Pneumonitis	Fever; cough; malaise; deep chest discomfort or pain; X-ray "shadows", usually patchy, may be diffuse. Complication of influenza, measles, chickenpox, zoster; rarely following coxsackie B virus. Rare form of slowly progressive diffuse, interstitial pneumonitis caused by CMV (usually in infants).	*Mycoplasma pneumoniae*; influenza A, influenza B, adenovirus, measles, Q-Fever, psittacosis, CMV. Infants: RSV and parainfluenza 3, other parainfluenza; adenovirus (types 1,3,7) assoc. with severe pneumonia in young children.
Q-Fever, Psittacosis	Fever; malaise; variable course from moderate flu-like illness or atypical pneumonia to severe pneumonitis; some cases prolonged or recurrent episodes. Rarely, myocarditis, endocarditis, or hepatitis may occur following Q-fever.	*Coxiella burnetii* (Q-Fever), *Chlamydia psittaci*, *Chlamydophila pneumoniae* (TWAR)
Pleurodynia, Pleuritis, Pleuropericarditis	Sharp "catchy" pain in side of chest (accentuated by breathing or coughing). Fever; malaise; headache. Pleural and/or pericardial effusion may occur as complication of Coxsackie pleurodynia, virus pleurodynia or viral pneumonias. Effusion seen by X-ray. Abnormal EKG in pericarditis.	Group B Coxsackieviruses; Viral pneumonia agents; often nonviral or unknown cause
Human Pulmonary Syndrome (HPS) (previously called Acute Respiratory Disease Syndrome (ARDS)	Previous healthy person, prodrome typically 3-4 days (fever, myalgia, headache, dry cough, injected conjunctivae) followed by ARDS or progressive interstitial pneumonia requiring intubation and mechanical ventilation.	Hantavirus
Section #3 – EXANTHEMS		

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Measles (Rubeola)	Fever; coryza; red eyes; cough for 3-4 days before typical "red" measles rash; rash usually prominent, blotchy on face, generalized. Dx may be more difficult in mild or atypical cases.	Measles virus
Rubella (see also congenital disease)	Slight fever, little or no prodrome before measles-like rash. Rash less red and blotchy (usually lasts for 3 days); arthralgia of fingers, wrists (less often knees - 5-10% of cases).	Rubella virus
Roseola infantum	Leucopenia, sometimes marked. High fever for 3 days then transient generalized rubella-like rash as fever falls. Commonly occurs in children less than 4 years of age.	Human Herpes Virus type 6 (HHV6B). (HHV7 may also be a causative agent.)
Rubella-like exanthema	Rashes clinically similar to rubella with a more variable duration and variable arthralgia. (Often drug or nonviral related). Usually some signs or symptoms of primary infection in addition to rash.	Echovirus (esp. 4, 6, 9, 14, 16). Rarely coxsackie A9, coxsackie B viruses, RSV, Adenovirus, Rubella, Measles
Erythema infectiosum (Fifth Disease, Slap Face Fever) see also congenital	Common childhood disease with mild symptoms including a fine rash on cheeks. May be confused with rubella and atypical measles.	Parvovirus B19
Dengue (breakbone fever)	Sudden onset of fever (lasting 5-7 days); intense headache; retro-orbital pains; joint and muscle pains. Rash appears 3-5 days after onset. Leucopenia and lymphadenopathy are usual. Complications include prolonged fatigue and depression.	Dengue types 1 - 4
Colorado Tick Fever (CTF)	Acute fever, headache, malaise, muscle aches 4-5 days after tick bite; occasionally encephalitic signs or rash.	Colorado tick fever virus
Typhus Fever	Fever, headache, petechial rash.	*Rickettsia typhi/R. felis*

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Rickettsiosis, Rocky Mt. Spotted Fever (RMSF), Pacific Coast Tick Fever	Fever, headache, myalgias, malaise, petechial rash, tick bite or exposure to ticks	*Rickettsia rickettsii, R. philipii* and other Spotted Fever Group (SFG) Rickettsia
Human Monocytic Ehrlichiosis (HME) Human Granulocytic Anaplasmosis (HGA)	Similar to Rocky Mt. Spotted Fever and Lyme Disease however rash may (or may not) be associated. Transmitted by ticks.	*Ehrlichia chaffeensis* (HME) (tropism for leukocytes [monocytes, lymphocytes and neutrophils]); *Anaplasma phagocytophilum* (HGA) (tropism for granulocytes)
Section #4 - VESICULAR ERUPTIONS		
Herpes simplex, Herpes stomatitis	Stomatitis (ulcers in mouth and gums) in initial infections in infants and children. "Fever blisters" (painful blisters on lips, around nostrils, etc.) typical of recurrent infection. Genital lesions. Generalized spread may occur.	Herpesvirus type I Herpesvirus type II
Chickenpox	Fever; crops of small vesicles widely distributed ("itchy" but not painful). Severe forms may occur in newborn, patient on steroid therapy or immunosuppressed. Pneumonia is serious complication.	Varicella-zoster virus (VZV)
Zoster ("Shingles")	Pain and tenderness in localized areas along nerve pathway followed by outcropping of vesicular lesions. Usually asymmetrical and on lower chest, back or over eye on the forehead. Also found as generalized chickenpox lesions.	Varicella-zoster virus (VZV)

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Molluscum contagiosum	Multiple chronic shin nodules. Pearly pink or white papules with a prominent central pore. May produce herpes-like lesions in the moist genital area.	Molluscum contagiosum virus (MCV)
Misc. pox infections Vaccinia, Cowpox	Humans infected by exposure to infected cows. Localized pustular skin lesions, slight fever. One or more lesions usually appear on hand (thumbs, first interdigital cleft and forefinger, most susceptible).	Orthopoxviruses
Milker's Nodule	Localized nodular skin lesions usually markedly proliferative. Transmitted via skin abrasions exposure to infected cattle.	Parapoxviruses
Orf	Transmitted via skin abrasions exposure to infected sheep.	Parapoxviruses
Generalized Vesicular Eruptions, Kaposi's varicelliform eruption, Stevens-Johnson syndrome, Eczema herpetiformis	Generalized vesicular eruptions with the entire body covered with vesicular pustular or bulbous lesions, especially in patients with chronic eczema. HSV, VZV and vaccinia may be clinically similar to each other. Nonviral causes include drug eruptions.	Herpes simplex viruses Varicella-zoster Vaccinia
Herpangina	Vesicular lesions in mouth and/or throat breaking to form ulcers. Typically very small lesions in tonsillar area, back of throat and palate; rarely forward.	Coxsackievirus Group A (esp. types 2-10); Less often Group B

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Vesicular stomatitis and exanthem (hand, foot & mouth disease)	Sore throat, small vesicles and ulcers in throat; "rice-grain" blisters on hands and feet.	Coxsackievirus (esp. type A16)
Section #5 - V. CONGENITAL INFECTIONS		
Congenital rubella syndrome	Varied defects including deafness, eye-defects, microophthalmia, heart defects, thrombocytopenia with purpura, syndactylism, bone defects, mental retardation, neonatal pneumonitis.	Rubella
Cytomegalic Inclusion Disease	Microcephaly, mental retardation, convulsions, motor disabilities, hearing loss; hepatosplenomegaly, neonatal hepatitis, pneumonia; inclusions in urinary epithelial cells.	Cytomegalovirus
Herpes simplex	Congenital defects when fetus is infected; often fatal generalized infection or permament brain damage, when baby is infected during birth.	Herpesvirus type 1 and 2
Fetal hydrops/ fetal demise	Fetal anemia, leading to heart failure and death. Usually occurs during 1st half of pregnancy. If not embryocidal, teratogenic effects are absent or rare.	Parvovirus B19
Congenital Zika Syndrome	Microcephaly, decreased brain tissue including subcortical calcifications, ocular damage including macular scarring, congenital contractures, hypertonia.	Zika virus
Section #6 - PERINATAL INFECTIONS		

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
AIDS	Pediatric AIDS can be transmitted in utero, intrapartum or via breast milk. HIV laboratory results can be confusing to interpret do the presence of maternal antibody. Most infected infants become culture and PCR positive by 8 weeks; 95% become positive by 6 months.	HIV-1 and HIV-2
Hepatitis B	Chronically infected mothers can often transmit HBV to their babies during birth and sometime afterwards. At least one-third of these infants will become chronically infected posing a lifelong infection risk to their future sexual and household contacts.	Hepatitis B
Section #7 - HEPATITIS		
Hepatitis A	Fever; malaise; loss of appetite; nausea; weakness lasting several days to week followed by jaundice, dark urine; light clay-colored stools.	Hepatitis A virus
Hepatitis B	Anorexia, vague abdominal discomfort, nausea and vomiting, sometimes arthralgias and rash. Often progresses to jaundice. Fever absent or mild.	Hepatitis B virus
Hepatitis C		Hepatitis C
Hepatitis D	Defective, requires co-infection with HBV	Hepatitis D
Hepatitis E	Enteric form of hepatitis especially common in India	Hepatitis E
Section #8 - IMMUNE and LYMPHATIC DISORDERS		
Infectious Mononucleosis	Children: generally mild disease, some splenomegaly. Adults: fever, sore throat, lymphadenopathy (esp. posterior cervical) general fatigue and weakness.	Epstein-Barr (EBV) Adenovirus CMV

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Chronic fatigue	Chronic fatigue, headaches, recurrent sore throat, recurrent fevers, swollen lymph glands, inability to concentrate, some memory loss, sleep disorders.	No known specific agent.
Adult T-cell Leukemia HTLV associated myelopathy (HAM also Tropical Spastic Paraparesis)	Lymphadenopathy, hepatomegaly, splenomegaly, cutaneous lesions without severe itching or excoriation, some immune deficiencies. Slowly progressive lower extremity weakness and spasticity with variable senory changes and spinal cord demyelination.	HTLV-I
HTLV-II	Not yet linked with a specific clinical illness but antibodies are common in IV drug abusers.	HTLV-II
Acquired Immunodeficiency Syndrome (AIDS), AIDS Related Complex (ARC)	Acute Syndrome: fever, malaise, myalgia, arthralgia, headache, macular rash and lymphadenopathy. AIDS syndrome: Kaposi's sarcoma, malignancies (esp B-cell lymphomas), CNS disease, decreased CD4 lymphocytes and nonspecific manifestations of immunosuppression such as Pneumocystis carinii pneumonia (PCP), Mycobacterium avium-intracellulare, pneumonia, toxoplasmosis, herpes zoster, diarrhea, and cryptococcal meningitis. ARC syndrome: persistent generalized lymphadenopathy, oral candidiasis, fever and weight loss.	Human Immunodeficiency Virus (HIV) types I and II
Section #9 - GASTEROINTESTONAL		

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Epidemic Viral Gastroenteritis	Usually a self-limited mild disease with nausea, vomiting, diarrhea, abdominal pain, myalgia, headache, malaise, low grade fever . Symptoms usually last 24-48 hrs.	Small Round Structured Virus (SRSV) Grouped into Astro and Caliciviruses based on EM morphology. (Norovirus (formerly Norwalk) is a member of the calicivirus group)
Sporadic Viral Gastroenteritis	Severe gastroenteritis of infants and young children; diarrhea, vomiting, often with severe dehydration, occasional deaths in young age groups. Hospital outbreaks common. Can re-infect adults exposed to infected children. Estimated shed of 10^{12} particles/ml of stool.	Rotavirus

Signoff History of Previous Manual Versions

Ver.	Annual Review	Date	Author/Supervisor	Director
1.0	Adopted PRIOR TO	April 1993	DT	RWE – April 1993
2.0	Reviewed - Revised	9/30/2007	DC	CG –
3.0	Reviewed - Revised	4/14/2008	DC	CG
3.5	Reviewed - revised	9/05/2008	DC	CG
4.0	Reviewed - revised	12/04/2008	GC	CG
5.0	Reviewed - Revised	3/20/2009	JL / DC	CG
5.1	Reviewed – Revised	3/11/2010	DC	DS
5.2	Reviewed – no change	10/24/2010	DC	DS
5.3	Reviewed - Revised	2/7/2011	DC	CH
5.4	Revised	5/17/2011	DC	CH
5.4.1	Revised (minor housekeeping corrections)	8/4/2011	DC	CH
5.4.2	Revised (minor housekeeping corrections)	10/31/2011	DC	CH
5.4.3	Revised (minor housekeeping corrections)	1/27/2012	DC	CH
5.4.4	Revised (minor housekeeping corrections)	4/26/2012	DC	CH
5.4.5	Revised (minor housekeeping corrections)	4/26/2012	DC	CH
5.4.6	Revised (minor housekeeping corrections)	10/25/2012	DC	CH
5.4.7	Revised (minor housekeeping corrections)	3/13/2013	DC	DX

5.4.8	Revised	12/23/2013	DC	DX
5.4.9	Revised	10/27/2014	DC	DX
5.4.10	Revised	12/19/2014	DC	DX
5.4.11	Revised	7/1/2015	DC	DX
5.4.12	Revised	4/19/2016	PP	DX
5.4.13	Revised	7/1/2016	MS / PP	DX
5.4.14	Revised	12/31/2016	MS	DX
5.4.15	Revised	6/15/2017	MS/DC	CH
5.4.16	Revised	12/15/2017	MS	CH
6.0	Revised	7/01/2018	MS	CH