



VIRAL AND RICKETTSIAL DISEASE LABORATORY

GUIDELINES FOR LABORATORY SERVICES

Version	Date	Author/Editor	Laboratory Director
4.0	12/4/2008	D. Cottam/G. Cosentino	Dr. Glaser
<p><i>Brief Description of Changes:</i></p> <ol style="list-style-type: none"> 1) Updated manual to reflect current testing policies 2) Expanded description of testing algorithms to reflect new Mumps and Measles PCR assays 3) Testing TATs updated (Enterovirus PCR now 14 days instead of 28 and Dengue EIA now 30 days instead of 14) 4) Removal of Hepatitis testing, Parvo IFA, SLE EIA & IFA, Electron Microscopy, Parainfluenza & RSV EIA 5) LCM IgG IFA added (previously accidentally omitted) 6) Updated PHL addresses and phone and fax numbers 7) HIV-1 EIA replaced with HIV1/2plus O Combi EIA 8) Clarification of diagnostic vs non-diagnostic assays per CLIA requirements in <i>Table of VRDL Assays</i> 9) Updated changes regarding non-endemic arbovirus testing, including Ross River Virus. 10) California Encephalitis Project testing algorithm added 			

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GENERAL INFORMATION

Introduction

This is an informational guide for clinical and public health laboratory staff regarding the availability of diagnostic assistance 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

<http://www.cdph.ca.gov/programs/vrdl/Pages/default.aspx>

This site will be updated with the latest diagnostic assays and services, submittal forms and information on special projects

COMMONLY USED ABBREVIATIONS			
<i>term</i>	<i>Organizations</i>	<i>term</i>	<i>definition</i>
CDPH	= California Department of Public Health	PCR	= polymerase chain reaction
VRDL	= CDPH Viral and Rickettsial Disease Laboratory Branch	IF	= immunofluorescence assay (can be used for antibody or antigen detection)
DCDC	= CDPH Division of Communicable Disease Control Branch	EIA	= enzyme immunoassay
MDL	= CDPH Microbial Disease Laboratory Branch	Wb	= Western blot
VBDB	= CDPH Vector Borne Diseases Branch	Direct	= Direct antigen detection
LCS	= CDPH Laboratory Central Services	RFFIT	= neutralizing test for rabies antibody
LAU	= Local Laboratory Assistant Unit (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

History

The Viral and Rickettsial Disease Laboratory (VRDL) is the oldest state public health virus 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. Leadership passed to Dr. Richard Emmons in 1989; to Dr. Michael Ascher in 1994. Dr. Carol Glaser, our current Laboratory Director, assumed the leadership in 2002.

Mission Statement

The Viral and Rickettsial Disease Laboratory (VRDL) provides laboratory support, technical assistance, and research required for the diagnosis, investigation, and control of viral 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, State Departments of Public Health 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 in its field of expertise. 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 Viral Immunoserology and Molecular Diagnostic Section** performs antibody testing and nucleic acid detection for over 20 different infectious diseases such as influenza, herpes simplex, VZV, measles, mumps, rubella and arboviruses.
- **The Viral Isolation Section** is responsible for over 80 diagnostic tests including the isolation of enteric, respiratory and central nervous system viruses and also provides rapid detection of agents such as rabies virus, adenovirus, and gastroenteritis virus.
- **The Epidemiology Support Section** provides testing services on all aspects of the diagnosis, treatment, virology, immunology, and epidemiology of HIV infection. They also provide serological testing for suspect Hantavirus cases.
- **The Retrovirus Diagnostic Section** serves as a statewide reference laboratory for HIV and other retroviruses and provides extensive consultation to local PHL's and clinicians throughout the state. Research activities include the development of new viral assays and monitoring of HIV vaccine trials.
- **The Medical and Epidemiology Liaison** section coordinates all diagnostic specimens received by VRDL for testing and answers questions regarding test availability, samples collection and shipment and interpretation of our test results. Our clients include other branches of the Department, local public health laboratories, clinical laboratories, and physicians throughout the state. This section coordinates several statewide projects including the encephalitis project, West Nile Virus project, Sentinel Influenza Physician project, Severe Influenza like illness (SILS) and Pediatric Death projects.

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. 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 submission for services available from the 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 www.CAPHLD.org**

There are currently 38 approved local public health laboratories in California. Viral 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 direct detection. While not all laboratories are now equipped to perform all tests, services are continually being extended

Local Public Health Laboratories and their contact information.

JURISDICTION	MAILING ADDRESS	PHONE	FAX
Alameda	1000 Broadway, Suite 500, Oakland, CA 94607	510-268-2700	510-268-2709
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
El Dorado	931 Spring Street, Placerville, CA 95667	530-621-6113	530-626-4713
Fresno	1221 Fulton Mall, Fresno, CA 93721	559-445-3507	559-445-3580
Humboldt	529 I Street, Eureka, CA 95501	707-268-2179	707-445-7640
Imperial	935 Broadway, El Centro, CA 92243	760-339-4437	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	559-582-0927
Long Beach	2525 Grand Ave, Long Beach, CA 90815	562-570-4075	562-570-4070
Los Angeles	12750 Erickson Ave., Downey, CA 90242	562-658-1300	562-401-5999
Madera	14215 Road 28, Madera, CA 93638	559-675-7893	559-675-0478
Marin	920 Grand Avenue, San Rafael, CA 94901	415-499-6849	415-499-6855
Merced	260 East 15th Street, Merced, CA 95340	209-381-1297	209-381-1292
Monterey	1270 Natividad Road, Salinas, CA 93906	831-7554-636	831-757-9586
Napa / Solano	355 Tuolumne Street, Vallejo, CA 94590	707-553-5059	707-553-5658
Orange	1729 West 17th Street, Santa Ana, CA 92706	714-834-8385	714-834-7968
Pasadena	1845 N. Fair Oaks Ave #301, Pasadena, CA 91103	626-744-6011	626-744-6126
Placer	11475 C Avenue, Auburn, CA 94603	530-889-7205	530-889-7209
Riverside	4065 County Circle Drive, Riverside, CA 92503	90-935-85070	909-358-5015
Sacramento	4600 Broadway, #2300, Sacramento, CA 95820	916-874-9231	916-874-9432
San Bernardino	799 East Rialto Ave., San Bernardino, CA 92415	909-38-33000	909-383-3094
San Diego	3851 Rosecrans Street, 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	805-78-15507	805-781-1023
San Mateo	225 West 37th Ave. Rm 113, San Mateo, CA 94403	650-573-3499	650-573-2147
Santa Barbara	315 N. Camino Del Remedio, Santa Barbara, CA 93110	805-681-5255	805-681-4753

JURISDICTION	MAILING ADDRESS	PHONE	FAX
Santa Clara	2220 Moorpark Ave. Rm 204L, San Jose, CA 95128	408-885-4272	408-885-4275
Santa Cruz	1080 Emeline Ave., Santa Cruz, CA 95060	831-454-5445	831-454-4296
Shasta	2650 Breslauer Way, Redding, CA 96001	530-225-5072	530-22-55074
Sutter	1445 Circle Drive, Yuba City, CA 95993	530-822-7225	530-822-7223
Sonoma	3313 Chanate Road, Santa Rosa, CA 94504	707-565-4711	707-57-67839
Stanislaus	820 Scenic Dr., Modesto, CA 95350	209-5587-356	209-558-7531
Tulare	1062 S. K Street, Tulare, CA 93274	559-685-2684	559-685-2586
Ventura	2240 E. Gonzales Rd Suite 160, Oxnard, CA 93036	805-981-5131	805-981-2130
Yolo	137 N. Cottonwood, Woodland, CA 95695	530-666-8644	530-669-1411

VRDL Contact Information

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

Carol Glaser, D.V.M., M.D, Chief (510) 307-8613 Carol.Glaser@cdph.ca.gov		
Janice Louie, M.D., Medical Officer (510) 307-8567 Janice.Louie@cdph.ca.gov		
VRDL main phone # (510) 307-8575; main fax # (510) 8599		
Section / Special Project	Section Chief	Section Supervisors / Project Coordinators
Virus Isolation and Rabies Section	David Schnurr, Ph.D. (510) 307-8615 David.Schnurr@cdph.ca.gov	Hugo Guevara (510) 307-8565 Hugo.Guevara@cdph.ca.gov
Viral Immunoserology and Molecular Diagnostic Section	Sharon Messenger, Ph.D. (510) 307-8623 Sharon.Messenger@cdph.ca.gov	Christopher Preas (510) 231-4185 Chris.Preas@cdph.ca.gov
Retrovirus Section	Carl Hanson, Ph.D. (510) 307-8540 Carl.Hanson@cdph.ca.gov	Janice Diggs (510) 307-8927 Janice.Diggs@cdph.ca.gov
Epi Support Section	Haynes (Chip) Sheppard, Ph.D (510) 307-8538 Haynes.Sheppard@cdph.ca.gov	Kent Dupuis (510) 307-8759 Kent.Dupuis@cdph.ca.gov
Medical and Epidemiology Liaison Section	Janice Louie, M.D (510) 307-8567 Janice.Louie@cdph.ca.gov	David Cottam (510) 307-8585 David.Cottam@cdph.ca.gov
CA Encephalitis Project	Carol Glaser, DVM, MD (510) 307-8613 Janice.Louie@cdph.ca.gov	Shilpa Gavali Jani (510) 307-8608 Shilpa.Gavali@cdph.ca.gov

West Nile Virus Project	Carol Glaser, DVM, MD (510) 307-8613 Janice.Louie@cdph.ca.gov	Cynthia Jean (510) 307-8606 Cynthia.Jean@cdph.ca.gov
Paediatric Severe Influenza Project	Janice Louie, M.D (510) 307-8567 Janice.Louie@cdph.ca.gov	Maria Nevares (510) 307-8923 Maria.Nevares@cdph.ca.gov
Sentinel Providers; California Respiratory Project; and Severe Influenza-like Illness Projects	Janice Louie, M.D (510) 307-8567 Janice.Louie@cdph.ca.gov	Erica Boston (510) 307-8503 Erica.Boston@cdph.ca.gov

Other Contact Information

Other California Department of Public Health Branch Contacts:

Microbiology Disease Laboratory Branch	(510) 412-3700
Infectious Disease Branch	(510) 620-3434
<ul style="list-style-type: none"> • Veterinary Public Health Section • Vector Borne Disease Section 	<ul style="list-style-type: none"> (916) 552-9740 (916) 552-9730
Immunization Branch	(510) 620-3737
Office of AIDS	(916) 324-8441

Types of Service Provided.

The VRDL offers three levels of service depending on the type of submitter. The Laboratory Chief or Medical Officer must be consulted before accepting any specimens from any non-California submitter. The VRDL:

- Provides routine diagnostic laboratory services for certain counties (table #2).
- The VRDL 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 health jurisdiction that has one.)
- The VRDL 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.
- The VRDL may accept specimens from non-California submitters if testing is not available in the submitter's jurisdiction.

See table on the following page

HEALTH JURISDICTION	VRDL SERVICES CURRENTLY PROVIDED TO LOCAL HEALTH DEPARTMENTS
Alpine	Animal rabies and other viral services not provided by San Joaquin County PHL
Amador	Animal rabies and other viral services not provided by San Joaquin County PHL
Calaveras	Animal rabies and other viral services not provided by San Joaquin County PHL
Colusa	All routine laboratory services
Del Norte	All routine laboratory services
Glenn	Animal rabies and other viral services not provided by Shasta County PHL
Inyo	All routine laboratory services
Lake	<i>No routine service.</i> All viral services provided by Mendocino County PHL
Lassen	Animal rabies and other viral services not provided by Shasta County PHL
Mariposa	Animal rabies and other viral services not provided by San Joaquin County PHL
Modoc	Animal rabies and other viral services not provided by Shasta County PHL
Mono	Animal rabies and other viral services not provided by San Joaquin County PHL
Nevada	<i>No routine service</i> All viral services provided by Sacramento County PHL
Plumas	<i>No routine service.</i> All viral services provided by Butte County PHL
San Benito	All routine laboratory services
Sierra	Animal rabies and other viral services not provided by San Joaquin County PHL
Siskiyou	<i>No routine service.</i> All viral services provided by Shasta County PHL
Tehama	<i>No routine service.</i> All viral services provided by Shasta County PHL
Trinity	Animal rabies and other viral services not provided by Shasta County PHL
Tuolumne	<i>No routine service.</i> All viral services provided by San Joaquin County PHL
Yuba	<i>No routine service.</i> All viral services provided by Placer County PHL
all other health jurisdictions	<i>No routine service.</i> Reference services is available upon request of their local PHL.

Specimen Collection, Storage and Shipment Guidelines

- Note - In order to ensure accurate patient and specimen identification, the submitter is required to provide the following information:**
- 1) Patient Name or Patient ID (must also be written on the sample container)
 - 2) Date of Birth
 - 3) Date of Onset (if applicable)
 - 4) Type of Specimen(s)
 - 5) 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 Assays	Acute phase- ASAP (no later than 7 days). Convalescent phase 14-21 days after onset	2.5 – 5ml of clotted blood or 1-2.5 ml of serum	None	4°C / none or cold pack	4°C / none or cold pack
Stool for isolation or direct detection (PCR) <i>See note #1</i>	ASAP – not later than 7 days after onset	2-4 grams	None	None	4°C / none or cold pack
Rectal swabs for isolation or direct detection (PCR) <i>See note #2</i>	ASAP – not later than 7 days after onset	1 – 2 swabs	2-3 ml of viral transport medium (VTM)	4°C / cold pack	-70°C / Dry Ice
NP, throat & nasal swab, ET aspirates, bronchial washing	ASAP – not later than 5 days after onset	1 – 2 swabs	2-3 ml of viral transport medium (VTM) Note#7	4°C / cold pack	-70°C / Dry Ice
Cerebrospinal fluid (CSF)	ASAP – not later than 3 days	1 – 3 ml	None	4°C / cold pack	-70°C / Dry Ice
Biopsy tissue		As much as available	Sufficient to keep sample moist	4°C / cold pack	-70°C / Dry Ice
Autopsy tissue	ASAP – within 24 hrs of death	½” - 1” cube of each sample	None	4°C / cold pack	-70°C / Dry Ice
Vesicular lesion fluid, basal cells from skin lesions; - for isolation or direct detection	ASAP – before crusting stage	1 -2 swabs	2-3 ml of viral transport medium (VTM)	4°C / cold pack	-70°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 and punch biopsy specimens for Rickettsial testing	ASAP	1 eschar or punch biopsy	4°C in a sterile gauze pad dampened with sterile saline.	4°C / cold pac	4°C / cold pac
Urine	ASAP - within 7 days	10 – 40 ml	<i>See note #3</i>	<i>See note #3</i>	<i>See note #3</i>

For any sample not described above – please call the VRDL at (510) 307-8585 for a consultation

- ☛ Note #1 – A minimum of 4 and maximum of 10 stools should be submitted from gastroenteritis outbreaks where Norovirus is the suspected agent.**
- ☛ Note #2 – 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. 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 viral examination. VRDL reserves the option of not testing rectal swabs.**
- ☛ Note #3 – Urine specimens are no longer considered to be the “Specimen of Choice” for suspected cases of measles, mumps or rubella. CDC is now recommending throat or NP swabs for these agents. Urine samples may be submitted upon prior consultation with the VRDL. Storage and shipment conditions for urine samples that cannot be delivered to the VRDL within 48 hours of collection are specific to the agent suspected.**
 - CMV – equal mixture of 70 % sorbital is required to preserve the virus if sample must be frozen.
 - Measles and Rubella – Urine should be spun down and the cell pellet resuspended in 1 -2 ml of VTM if the sample must be frozen.
- ☛ Note#4 Testing Acute Blood Specimens - Acute specimens of particularly high public health significance for which reliable IgM tests exist (in particular measles, SLE/WEE, HPS or in cases of a Specimen Alert) are tested ASAP. Testing a single acute blood for other requests is generally not useful and a follow up specimen should be requested.**
- ☛ Note #5 Testing non-acute Blood Specimens - Single specimens from diseases with insidious onsets and convalescent specimens are tested if it is felt that the results will provide meaningful information to help patient management. Frequently a negative result is the most useful (such as a negative antibody result for herpes is a strong indication that the patient is not infected with herpesvirus).**
- ☛ Note #6 Testing CSF Specimens - CSF specimens can be tested for antibody, for virus isolation or both depending on a number of factors such as agent suspected, onset date relative to collection date and availability of test. If in doubt about the type of test, consult Dr. Glaser or Dr. Louie.**
 - CSF specimens taken within a few days of the date of onset and shipped promptly at 4⁰C via an overnight delivery service or 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 and will be routed for isolation attempts if above conditions are met.
 - CSF specimens which do not meet the criteria above but has a corresponding blood specimen are held pending the outcome of the serology results on the blood sample. If no antibody is detected in the blood specimen, the CSF is not tested. If antibody is detected in the blood specimen, the CSF specimen is tested for that antibody.
- ☛ Note #7 – To preserve the infectivity of NP, nose and/or throat swabs use 1-2 ml of Viral Transport Medium (VTM) to protect the swab. 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 viral examination. VRDL reserves the option of not testing rectal swabs.**

VRDL Specimen Submittal Forms

The VRDL has a variety of specimen submittal forms – many customized to provide specific specimen collection instructions, obtain specific epidemiological information, and clinical signs and symptoms to help us evaluate your patient's illness and provide you with the best possible laboratory support.

Examples of these specimen submittal forms include:

- Avian (or other pandemic) Influenza
- California Encephalitis Project
- California Respiratory Project
- Culture for Identification
- Gastroenteritis Outbreaks – Suspected Norovirus
- Gastroenteritis Outbreaks – RNA extracts from positive stools
- Hantavirus – human pulmonary syndrome
- Hepatitis C
- Influenza and other Respiratory Illness
- Influenza Strain Typing
- Pediatric Severe Influenza
- Rabies (animal)
- Sentinel Providers for Respiratory Surveillance Project
- West Nile Virus Project
- VRL300 – Our standard specimen collection form

 **These forms change frequently and so no attempt will be made to include them in this manual. The most up-to-date versions of these forms are available in PDF format on our VRDL website <http://www.cdph.ca.gov/programs/vrdl/Pages/default.aspx>.**

Shipment of Clinical Samples

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.

 **Note – 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. You should assume that the package will go by air unless you know it will be delivered by ground transport.**

Unregulated - Samples known not to 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)

A Category A substance or agent is one that is capable of causing permanent disability, 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
- A Shipper's Dangerous Goods Declaration must accompany the package
- A 24/7 contact phone number must be provided in case the package leaks during transportation.

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

- DOT website -http://hazmat.dot.gov/training/Transporting_Infectious_Substances_Safely.pdf
- ICAO website - <http://www.iata.org/NR/rdonlyres/B8B91553-49BE-4DCC-901B-50DAE57A98E/0/GuidanceDocument18Nov.pdf>
- IATA website – <http://www.iata.org/NR/rdonlyres/759F5AF2-165A-4DDB-8899-2F2C8DE4797F/0/Section362200.pdf>

Shipping Containers

Note - Because of the complexity of packaging requirements, the VRDL no longer routinely provides shipping containers.

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 must be approved by the VRDL Chief, VRDL Medical Officer or the Acting VRDL Chief

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 under Special Instructions “**RING BELL AT GATE FOR ADMITTANCE**”.
 - Golden State Overnight has an early Saturday morning delivery by 8:00 AM.
 - Upon prior consultation, 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 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.
- VRDL staff will send an e-mail to the FMS Duty Officer who will alert the security guards to watch for the package.
- Whenever possible, once the faxed copy of the waybill is received, VRDL staff will call the transportation company and notify them that the laboratory is expecting this package and assure the company that there will be someone at the laboratory to receive it.

Requests for Laboratory Results

Note: Results may only be reported to the original submitting laboratory, health department or patient's physician. 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 mailed to the submitter.

In cases of high public health importance or where time is of the essence, laboratory results are reported verbally or by fax. The person requesting a fax must guarantee that the fax machine is in a secure, non-public location.

If the laboratory results are still pending and a reasonable time has elapsed, please call the VRDL at (510) 307-8585. Our 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 Chief or Medical Officer if you believe that you are sending the VRDL samples that may contain unusually hazardous agents. The VRDL Chief (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 and possible BT agents (such as “white powder”).

 **Note - Special shipping rules and regulations apply to shipping Select Agents**

POLICIES GUIDELINES AND TESTING ALGORITHMS

Arbovirus –

- ☛ **NOTE: Members of the Group B arbovirus (including SLE, Dengue, and Yellow Fever) usually produce antibodies that cross-react with one another. Therefore a knowledge of prior immunization (i.e. for yellow fever) and travel history is important for the proper interpretation of test results.**
- ☛ **NOTE: Members of the Group A arbovirus (including WEE and CHIK) often produce antibodies that cross-react with one another.**

Arboviruses endemic in California

EIA is performed for WEE on bloods depending on the mosquito activity for that year or if the patient has a travel history to an endemic area during their mosquito season. Samples are screened for IgG antibodies by EIA.

West Nile Virus (WNV) – Requests for WNV are coordinated by the WNV Special Project as follows:

If only serum is received, the sample is tested for EIA IgG and IgM

If only CSF is received, the sample is tested for EIA IgM and Enterovirus PCR

if both serum and CSF are received, the serum is tested for EIA IgG and IgM and the CSF sample is tested for Enterovirus PCR.

Additional testing such as IFA or PRNT may be done in the following situations:

- If only one positive result is obtained, e.g. EIA IgM(+), EIA IgG(-) – IFA may be requested, or if both a serum and CSF were received, the CSF may also be routed
- To expedite turnaround for “high-profile” cases e.g. very ill patient or first case for county – IFA may be requested
- For discrepant results or for patients with travel history, additional testing e.g. PRNT or other arbovirus EIA may be requested

Arboviruses not endemic in California

Requests for arboviruses not endemic in California, such as California Encephalitis Virus (CEV), Dengue (DENG), Chikungunya (CHIK), Ross River Virus and Yellow Fever (YF) are tested as follows:

California Encephalitis Virus (CEV) is not offered as a routine service since this virus is rare California. Requests for CEV will be sent to CDC for testing.

Chikungunya (CHIK) - Patient should have a travel history to an endemic area (such as Southern Asia). If you not sure an area is endemic for dengue consult with your Health Officer or the VRDL Medical Officer for clarification

Samples are tested for dengue, WEE and CHIK. IgG antibody and reflexed for IgM testing if IgG is detected.

Until the VRDL CHIK is validated, these samples are also sent to CDC at Ft. Collins for more specific testing. Until our CHIK tests are validated, only CDC’s results for CHIK will be reported to the submitter. Samples can be forwarded to CDC for additional testing (serology, PCR and virus isolation).

Arboviruses not endemic in California, cont.

Dengue (types 1-4) - Patient should have a travel history to an endemic area (Mexico, Caribbean, Tahiti, Southeast Asia and India, etc.). Routine testing is by IFA for IgG and IgM antibodies. Note: This assay does not distinguish between types. CDC can perform strain typing on very acute serum in cases of special public health interest.

Dengue request (no travel history to countries with endemic CHIK)

Sample is tested for dengue IgG antibody and reflexed for IgM testing if IgG is detected.

At the current time, these samples are also sent to CDC at Ft. Collins for more specific testing.

Dengue request (with travel history to countries with endemic CHIK)

Sample is tested for dengue, WEE and CHIK. IgG antibody and reflexed for IgM testing if IgG is detected.

At the current time, these samples are also sent to CDC at Ft. Collins for more specific testing.

Ross River Virus is a Group A arbovirus found in the South Pacific and Australia. Cross-reactions among Group A arboviruses are problematic and VRDL does not offer any routine service. Samples will be forwarded to CDC which has specific reagents for this virus.

Yellow Fever is not offered as a routine service and VRDL and requests for YF will be sent to CDC for specific testing. Specimens may be tested for SLE which often cross-reacts with the yellow fever virus.

Chlamydia

There are three species in this genus that causes human disease. *C.psittaci* is transmitted by exposure to infected birds. *C.pneumoniae* is associated with primary atypical pneumonia in young adults. *C.trachomatis* has two biovars. Lymphogranuloma venereum (LGV) biovar is more invasive and associated with lymphoid tissue. The Trachomatis biovar causes eye disease which can lead to blindness.

There is extensive cross-reactivity between the three species. VRDL antibody tests for IgG and IgM do not distinguish between the three different species and the causative agent is determined by the clinical presentation.

Samples from suspected cases of *Chlamydia* pneumonia of the newborn will be forwarded to CDC for IFA IgM which is the test of choice.

VRDL does not offer isolation for the Chlamydia agents.

Encephalitis – The VRDL has been involved in a project, the California Encephalitis Project, to identify the etiology of encephalitis in hospitalized California residents for which the causative agent is unknown. A comprehensive battery of testing is done on serum, CSF, and respiratory specimens. A separate population based project has been initiated in 2007 in order to enroll all hospitalized patients within the Kaiser Permanente system. For more information about either project, please visit the Project's website (www.ceip.us) or call (510) 307-8608.

Testing algorithm

A. CSF (need 2-3 ml)- ***If lumbar puncture is not performed, CSF is not needed***

1. PCR for HSV 1 & 2; VZV; HHV6, Enterovirus
2. Freeze aliquot for further testing
3. Further testing as patient history indicates

B. Serum (1 red top tube or 2-3 ml) -

1. Mycoplasma IgM; EBV IgM, IgG, EBNA
2. Additional testing as patient history or seasonality indicates

- C. NP/Throat swab (in viral transport media) –
 1. PCR for Enterovirus
 2. PCR for Rhinovirus
 3. PCR for *Mycoplasma pneumoniae*
 4. Routine viral culture
 5. Respiratory PCR panel if patient has URI symptoms
- D. Convalescent serum (10-14 days after onset or date of acute serum) -
 1. Mycoplasma IgM, Mycoplasma IgG
 2. Further testing as patient history indicates
 3. Additional testing per results of acute specimens
- E. If PCR is positive, follow-up serology for particular agent
- F. Consider rabies if severe illness, travel history outside of US, or rapid progression of illness.

Gastroenteritis Samples

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

For Local HDs that can not perform Norovirus PCR testing

A Norovirus specific case history and group submittal and instruction form is available on the VRDL website or can be faxed upon request.

- Note that while vomitus may contain high norovirus titers, our PCR has only been standardized 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 should be discussed with laboratory personnel before tests are conducted.

Number of Samples – CDC requires a minimum of two (2) positive for Norovirus before they will consider Norovirus to be the causative agent for the GI outbreak. Thus for meaningful laboratory results (see interpretation below) specimens from a minimum of four (4) and preferably more, up 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 (up to a maximum of 10), the more meaningful the test results.

- Notes -A single stool sample will not be tested since neither a positive nor negative result will be meaningful.**
- 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 4⁰C 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 should be frozen if they cannot be shipped to the laboratory within 3 weeks.

Interpretation of Test Results

- **Note: Norovirus PCR testing is intended for use primarily as laboratory support for epidemiological investigations.**

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.
- **INCONCLUSIVE** – All other outcomes.

For Samples Positive for Norovirus PCR testing at the Local HD

Local HDs are strongly encouraged to submit two positive RNA –extract 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 circulation 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 agents 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. 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.**

If the patient does not meet the CDC case definition or you would like a consultation, call Dr. Janice Louie at the Viral and Rickettsial Disease Laboratory at (510) 307-8567 or Dr. Curtis Fritz, Vector-Borne Disease Section, at (916) 552-9730. If Dr. Louie or Dr. Fritz is not available, local health departments may contact the Duty Officer at (510) 620-3434.

Clinical consultations for patient management are available from the staff at the University of New Mexico Medical School. Call 1-888-UNMPALS and request a HPS consultation.

Specimen Submittal Instruction- Fill out the HPS case history form as completely as possible. Fax one copy to David Cottam at (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.
- **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 -70oC).
- NP swab and/or lower respiratory sample (such as an ET aspirate or bronchial wash).

In cases where our HPS results are equivocal or inconsistent with the clinical presentation, specimens may be forwarded for further testing to either the Centers for Disease Control and Prevention or a reference laboratory at the University of New Mexico.

Hepatitis

Hepatitis A, B and C These tests are widely available at commercial labs, community hospitals and some local public health laboratories. Hence, requests are only offered in support of DCDC epidemiological investigations and not offered as a routine service.

IgM Antibody Requests.

IgM can be performed on many viruses if warranted by special public health circumstances and prior consultation with the VRDL Laboratory Chief or Medical Officer. Testing for Mycoplasma IgM is routinely offered. All other requests for IgM antibody testing is only performed for viruses of unusual public health significance (such as measles) or in cases where an IgG results indicates that IgM testing is warranted.

Immunity Status Requests

Requests for immunity testing is not a routine service. Exceptions are made 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 status 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 Fluorescent Foci Inhibition Test (RFFIT) which measures neutralizing antibody. This test is labor intensive and is currently only performed once every three (3) months. Do to the limited numbers of samples that can be tested, prior approval is required for all non-public health laboratory staff.**
- Note: This test is performed at the Kansas State University on a weekly basis at a very reasonable cost. Information and submittal forms can be obtained from their website: www.vet.ksu.edu/depts/dmp/service/rabies/index/htm or by telephone (785) 532-4483.**
- Varicella for special situations of unusual public health importance (such as hospital situation where an immediate answer is necessary for staff or patient management)
- For suspected cases of chickenpox - DFA on smears prepared from lesion basal cells provides rapid laboratory results**

Infectious Mononucleosis (Epstein Barr Virus EBV)

The VRDL offers serology testing for VCA IgG, VCA IgM and EBNA antibodies. This combination of tests usually provides a good indicator of when a patient was infected. Note: Although the Monospot test is not offered by VRDL. it is a relatively reliable, nonspecific test for infectious mononucleosis and should be done by submitter before sending samples to the VRDL.

- The Monospot can produce a false negative in approximately twenty percent of patient under 25. Therefore specimens from patients with a negative Monospot that have symptoms compatible with infectious mono illness, Burkett's lymphoma, or other problem diagnosis may be submitted for EBV specific testing.**

Under 6 years of age should be done more readily following the CDC's policy on this age group

Rabies virus

Rabies (Human) – Requests for cases of suspected human rabies are referred to the Encephalitis Project Coordinator for follow up. Samples vary depending on the clinical status of the patient.

Rabies – (Animal) Frequently the LAU is notified by a local health department that they are shipping a brain specimen from an animal involving a significant human exposure. In these cases, LAU staff will initiate an e-mail notification to Dr. Schnurr, Isolation Supervisor, and the microbiologist on schedule to perform the rabies testing.

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 Chief, VRDL Medical Officer or the Acting VRDL Chief.

In general, the VRDL will call back a microbiologist to perform rabies testing on weekends or holidays under the following conditions:

- The test is to evaluate a suspected case of human rabies or
- A treatment decision is pending based on the laboratory result. The VRDL Chief, VRDL Medical Officer, Acting VRDL Chief or a State Veterinarian must determine that testing cannot be postponed until the following business day.
- The laboratory is notified as early as possible on Friday but no later than 4:30 PM that such a specimen is expected. This will allow for notification of the microbiologist and security guard that a specimen is expected.
- A shipment method is utilized that will guarantee that the specimen will arrive as scheduled.

🔗 It is especially important that prior arrangements are made to ensure that the submitter receive the laboratory results. See Page 14 for Instructions.

Respiratory Samples

🔗 Note - Often there are specific submittal forms and instructions for each of the projects described below. Please visit the VRDL website and download the appropriate form.

Sentinel Providers Respiratory Surveillance Project – Erica Boston

- NP or throat & nasal swabs routed for Virus Isolation in R-mix
- If Influenza virus is isolated, strain typing will be performed.

Respiratory Outbreaks & Special Requests – LAU staff

- Isolation samples routed simultaneously for Respiratory PCR panel #1 (Influenza A & B, Adenovirus, RSV and human metapneumo viruses, parainfluenza (PIV) types 1→4).
- If Respiratory PCR panel #1 is negative, samples may be tested by PCR for additional respiratory agents (Mycoplasma and Rhinovirus) or other tests indicated by Medical Review.
- If Influenza virus is isolated, strain typing will be performed and antiviral resistance testing if indicated by Medical Review.

Samples Received from Local Health Departments - LAU staff

- Isolation samples routed for Virus Isolation only
- If Influenza virus is isolated, strain typing will be performed and antiviral resistance testing if indicated by Medical Review.

Pediatric Severe Influenza cases- Maria Nevares

- Isolation samples routed simultaneously for Respiratory PCR panel #1 (Influenza A & B, Adenovirus, RSV and human metapneumo viruses, PIV 1 and 3) and Virus Isolation
- If Respiratory PCR panel #1 is negative, samples may be tested by PCR for additional respiratory agents (PIV 2 and 4, Mycoplasma and Rhinovirus) or other tests indicated by Medical Review.
- If Influenza virus is isolated, strain typing will be performed and antiviral resistance testing if indicated by Medical Review.

California Respiratory Project - Erica Boston

- Isolation samples routed simultaneously for Respiratory PCR panel #1 (Influenza A & B, Adenovirus, RSV and human metapneumo viruses, PUIV 1 and 3) and Virus Isolation
- If Respiratory PCR panel #1 is negative, samples may be tested by PCR for additional respiratory agents (PIV 2 and 4, Mycoplasma and Rhinovirus) or other tests indicated by Medical Review.
- If Influenza virus is isolated, strain typing will be performed and antiviral resistance testing if indicated by Medical Review.

Severe Illness Laboratory surveillance (SILS) - Erica Boston

- Isolation samples routed simultaneously for Respiratory PCR panel #1 (Influenza A & B, Adenovirus, RSV and human metapneumo viruses) and Virus Isolation
- If Respiratory PCR panel #1 is negative, samples may be tested by PCR for additional respiratory agents (PIV 2 and 4, Mycoplasma and Rhinovirus) or other tests indicated by Medical Review.
- If Influenza virus is isolated, strain typing will be performed and antiviral resistance testing if indicated by Medical Review.

Suspected Avian Influenza (or other pandemic) -LAU staff

- Samples containing suspected Avian (or other pandemic) Influenza virus will be screened by real time PCR for Influenza A H1, Influenza A H3 and Influenza H5.
- If samples are negative for Avian (or other pandemic) Influenza viruses, routine virus isolation will be attempted.

CA Encephalitis Project (with respiratory illness) - Shilpa Gavali Jani

- Isolation samples routed simultaneously for Respiratory PCR panel #1 (Influenza A & B, Adenovirus, RSV and human metapneumo viruses) and Virus Isolation
- If Respiratory PCR panel #1 is negative, reflex to Mycoplasma and Rhinovirus PCR and other tests indicated by Medical Review.
- If Influenza virus is isolated, strain typing will be performed.

Retrovirus Samples***AIDS Serology***

- All specimens sent to our laboratory for HIV testing are screened by Enzyme Immunoassay (EIA) and Immunofluorescence Assay (IFA) simultaneously.
- We use the licensed Bio-Rad HIV-1/HIV-2 PLUS O EIA kit, an in-house HIV-1/HIV-2 IFA test, and the licensed Bio-Rad HIV-2 EIA kit.
- When HIV EIA and IFA results agree an overall interpretation of “Antibody Detected” or “Antibody Not detected” is reported.
- When HIV EIA and IFA results are discordant or the IFA is unsatisfactory (nonspecific), HIV-1 and/or HIV-2 Western blot (WB) is performed.

HIV-2

- Travel history or exposure history support HIV-2 request is required prior to start of testing.

HTLV

- All samples are screened by EIA and IFA
- Positives are 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.
- If two of the above tests do not agree, sample is reflexed for RIPA testing

HTLV Overall Interpretation - The overall HTLV interpretation is determined by the results of all tests performed. Two assay methods must agree before we report the results of our laboratory tests.

- Antibody Detected – antibody detected by at least two of the following three assays – EIA, IFA and/or Western blot.
- Antibody Not Detected – at least two of the our three assays (EIA, IFA and Western blot) did not detect HIV antibodies
- Inconclusive – all test results are
- Unsatisfactory - sample was nonspecific or inappropriate for testing

Western blot interpretations require at least the following bands:

Positive p19 or p24 plus p21e bands

Indeterminate p21e band only

Negative p21e band is absent. Regardless of Core bands (p19 and/or p24)

Rickettsial Agents

Rickettsia Agents Endemic in California

Rickettsial Agents (RMSF, Typhus, Ehrlichia and Q fever) are endemic in California. Since symptoms can overlap, if Rickettsial testing is requested, we routinely screen for all of these agents.. Epidemiologic information (including travel history and any know exposures to ticks, fleas, lice etc) is required.

Q Fever Serology Q fever Phase 2 is the normal antigen used in the IF test to detect acute infection with this agent. Specimens that are positive for Phase 2 are then further tested for Phase 1 which is an indicator of possible endocarditis.

Rickettsial agents are very difficult to grow and isolation attempts are not offered by this laboratory. Heart tissue for Q fever can be examined by EM if prior arrangements have been made and can be forwarded to the CDC for PCR if necessary.

Note: Q fever is extremely infectious and all samples suspected of containing this organism should be handled with special care unless the sample has been inactivated (i.e. treated with formaldehyde)

- Sample requesting serology for RMSF, Typhus and/or Ehrlichia and Q fever will be routed to the IFA Unit for IgG testing for all four agents.
- If positive, reflex for IgM testing.

Rickettsia Agents Not Endemic in California

Currently VRDL is performing testing for newly identified rickettsial agents that may be present in California on a research/surveillance basis. These new agents include *Rickettsiae felis* (identified in opossums in Southern California) and *Rickettsiae phillipi* (newly identified in humans in Lake county). Other agents of interest include *Rickettsiae africae* and *Rickettsiae conorii* in travelers. Possible testing will include IFA, PCR, isolation and referral to CDC for immunohistochemistry of tissue.

Samples requesting serology for which the VRDL does not have specific reagents will be sent to CDC in Atlanta for specific testing.

- Note – Depending on the known cross-reactivity of the agent requested, the VRDL may route the sample to the IFA Unit to test for a related agent.**

Samples Requested and Storage Conditions

To assist with surveillance studies, for all cases where ANY rickettsial infection is suspected, the following samples should also be requested (as available):

- Acute and convalescent sera (5-10 cc) collected in a red top or tiger top tube
- Acute phase whole blood (5-10 cc) collected in an EDTA purple top tube
- Eschar (if present)
- Punch biopsy of rash (if eschar not present)- obtain using a 2.5-6.0 mm skin punch
- Swab of open lesions, pustules or vesicles collected in sterile saline

Eschar and punch biopsy specimens should be stored at 4°C in a sterile gauze pad dampened with sterile saline until they are shipped to VRDL (preferably overnight, or within a day or two). Upon arrival at VRDL, the specimen should be split; half should be frozen at -70°C and the other half should be fixed in 10% formalin.

Vaccine Preventable Diseases

Measles virus is no longer endemic in the United States so CDC is especially interested in identifying the strain type of measles cases to track their origin. This test requires isolation of the virus when measles is suspected, it is especially important to collect samples for isolation. A throat swab is the best source of virus in the early infectious stage. Although measles can sometimes be isolated from urine later in the infection, **we are no longer requesting urine as a specimen of choice.**

Measles Isolation – The availability of a sensitive cell line (B95a) for measles isolation has greatly increased our ability to isolate. CDC can then use molecular epidemiological techniques to identify the source of the wild-type viruses and rapidly differentiate between wild-type and vaccine strains. Respiratory Samples taken within no later than four (4) days after rash onset are the best source to recover infectious virus. A nasal aspirate is the preferred sample but alternatively, a nose and throat swab should be taken and placed in a single vial containing 2-3 ml of VTM. Samples which can be received by the VRDL within 48 hours should be stored and shipped at 4°C. Else, the nasal aspirate should be centrifuged at 2500 x g for 15 minutes at 4°C and the pellet resuspended in 1 ml of VTM, then stored and shipped at -70°C or colder. Nose and throat swabs should be stored and shipped at -70°C or colder.

- **Urine Samples – Not Routinely Recommended – Call VRDL for a consultation before collecting or submitting a urine sample.** If VRDL recommends that a urine be collected then collect up to 50-100 ml of urine within the first week after rash onset. Process by centrifuging at 2500 x g for 15 minutes at 4°C. Resuspend the pellet in 1-2 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 4°C by overnight delivery service.

Measles Serology - The VRDL can test for both measles IgG and IgM antibodies. The gold standard is the detection of a significant change between the acute and convalescent serum samples. IgM related to a current infection with measles virus can usually be detected approximately 70% of the time in an acute sample taken at least 3 days after rash onset. Confidence increases to approximately 99% in a sample taken 7 days after rash onset. Serology specimens should be collected in red top tubes (5 cc is ideal but 1-2 cc is acceptable) for optimal results. However, in cases where collection of specimens may be difficult (e.g., infants), VRDL can attempt testing of specimens collected in capillary tubes, although this is not optimal. Capillary tubes should be capped and placed in another larger tube for protection before transport. The specimens should be spun so that sera are separated out. Because the volume of specimens is extremely low in capillary tubes, multiple samples should be collected and VRDL cannot

guarantee that enough specimen will be available for supplementary testing or forwarding to CDC for reference testing or stain typing.

Measles PCR - The VRDL offers PCR for testing respiratory samples for measles virus.

- **Note taken from the CDC VPD Surveillance manual - 10% of recipients of measles-containing vaccine may develop fever and rash approximately one week after vaccination, and vaccination of susceptible persons results in the production of IgM antibody that cannot be distinguished from the antibody resulting from natural infection. A positive measles IgM test cannot be used to confirm the diagnosis of measles in persons with measles-like illness who received measles vaccine 6-45 days before onset of rash. A negative test would exclude the diagnosis.**

Mumps

Serology - VRDL currently performs an IFA assay to measure mumps IgM antibodies but results must be interpreted with caution since false positive or non-specific reactions are known to occur. The gold standard remains the demonstration of a significant change in titer between the acute and convalescent samples.

Isolation can be attempted from throat swabs collected within 9 days of onset of parotitis. **Urine samples are not routinely recommended – Call VRDL for a consultation before collecting or submitting a urine sample.**

Mumps PCR – The VRDL offers PCR testing on respiratory samples for Mumps Virus.

Rubella –

Isolation can be attempted from throat swabs collected within 7 days of onset of symptoms. Please call the laboratory if suspected congenital rubella is suspected. **Urine samples are not routinely recommended – Call VRDL for a consultation before collecting or submitting a urine sample.**

Serological testing for rubella IgM antibody is available but it should be noted that there is potential non-specific cross-reaction with parvovirus IgM. The gold standard is to the detection of a significant change between the acute and convalescent serum samples.

Polio - Neutralization test. No routine service. Testing may be performed at the request of DCDC for patients with special circumstances.

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

Varicella

Varicella Direct Detection - When necessary, isolation or direct detection of varicella virus is best accomplished by removing the cap or scab and collecting cells from the base of the lesion on a swab. Use the swab to make a smear on a microscope slide (for direct detection) and then place the swab into 1-2 ml of VTM (for isolation attempts).

The cap or scab may also be submitted in a clean, sterile container. These samples can be examined by electron microscopy.

Varicella Serology – Significant rise in varicella IgG antibody levels between acute and convalescent serum.

“White Powder”

The Microbial Diseases Laboratory (MDL) has taken on the responsibility for screening “white powder” samples.

- ✎ **Call the Microbial Disease Laboratory at (510) 412-3700 for a prior consultation so that special arrangements can be made for receiving, handling and testing these sample. Special arrangements may include Chain of Custody (COC) requirements.**

Referring Samples to Local HD, MDL and/or CDC

VRDL staff may refer samples to other laboratories upon review by the Laboratory Chief, VRDL Medical Officer or acting Chief. Samples are routinely referred to another laboratory if

- The sample was mistakenly addressed to the VRDL
- 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 Centers for Disease Control (CDC)
- The test requested is performed by MDL

APPENDIX

Appendix A Table of VRDL Assays – Sorted by Agent

Updated: December 4, 2008

- The table below shows VRDL assays that are currently validated per CLIA requirements and unless otherwise specified, are routinely available. This table is subject to frequent change as new assays are developed and validated. Please visit the VRDL Website for the latest table update.
- Note: a status of “non-diagnostic” means that the assay is performed for surveillance purposes or when authorized by the Laboratory Director (or designee) for special circumstances.
- Note: 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
- Note: 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 from VRDL Chief, VRDL Medical Officer or acting Chief is required when requesting expedited testing.

VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 11/18/2008	ASSAY	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT	SAMPLE
Arboviruses						
Chikungunya	Antibody	Blot	Non-diagnostic	Travel history required	30-60 days	
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)	Neutralization	PRNT	Non-diagnostic		28 days	serum
Dengue (does not distinguish type)	Antibody	Blot	Non-diagnostic		14 days	serum
St. Louis Encephalitis SLE	Neutralization	PRNT	Non-diagnostic		28 days	serum
St. Louis Encephalitis SLE	Antibody	Blot	Non-diagnostic		14 days	serum
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	Antibody	Blot	Non-diagnostic		14 days	serum
West Nile Virus WNV	Isolation	Cell Culture	Non-diagnostic			
West Nile Virus WNV	Neutralization	PRNT	Non-diagnostic		28 days	serum

VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 11/18/2008	ASSAY	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT	SAMPLE
Western Equine Encephalitis WEE	IgG	EIA	Diagnostic	Clinical and case history required	14 days	serum
Western Equine Encephalitis WEE	Neutralization	PRNT	Non- diagnostic		28 days	serum
Western Equine Encephalitis WEE	Antibody	Blot	Non- diagnostic		14 days	serum
Adenoviruses	IgG & IgM	EIA	Diagnostic		14 days	serum
Adenoviruses	IgG & IgM	IFA	Non- diagnostic			serum
Adenoviruses	Isolation	Cell culture	Diagnostic		28 days	Respiratory
Adenoviruses	Direct Detection	Real Time PCR	Diagnostic		14 days	Respiratory
Chlamydia (does not differentiate between psittaci, LGV or pneumoniae species)	IgG & IgM	EIA	Diagnostic		14 days	serum
<i>Coxiella burnetii</i> (Q fever)	Phase II IgG	IFA	Diagnostic		14 days	serum
<i>Coxiella burnetii</i> (Q fever)	Phase I IgG	IFA	Diagnostic		14 days	serum
<i>Coxiella burnetii</i> (Q fever)	Phase II IgM	IFA	Diagnostic		14 days	serum
Cytomegalovirus (CMV)	IgG & IgM	EIA	Diagnostic		14 days	serum
Cytomegalovirus (CMV)	Direct Detection	Real Time PCR	Diagnostic		21 days	CSF
Cytomegalovirus (CMV)	Isolation	Cell Culture	Diagnostic		21 days	Respiratory or urine
<i>Ehrlichia chaffeensis</i>	IgG & IgM	IFA	Diagnostic	Case history required	14 days	serum
<i>A. phagocytophilia</i> (HGE)	IgG & IgM	IFA	Diagnostic		14 days	serum
Enteroviruses	Isolation	Cell Culture	Diagnostic		28 days	respiratory or fecal
Enteroviruses	IgM	EIA	Non- diagnostic		14 days	serum or CSF
Enteroviruses	Direct Detection	Real Time PCR	Diagnostic		14 days	CSF
Enteroviruses	IgG	Serum Neut	Non- diagnostic	Prior consultation required	30 days	Acute and Convalescent serum required
Epstein-Barr Virus (EBV)	VCA IgG	IFA	Diagnostic		14 days	serum
Epstein-Barr Virus (EBV)	VCA IgM	IFA	Diagnostic		14 days	serum

VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 11/18/2008	ASSAY	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT	SAMPLE
Epstein-Barr Virus (EBV)	EBNA	IFA	Diagnostic		14 days	serum
Epstein-Barr Virus (EBV)	Direct Detection	Real Time PCR	Diagnostic		21 days	CSF
Herpes simplex virus (HSV) (does not distinguish type)	IgG & IgM	EIA	Diagnostic		14 days	serum
Herpes simplex virus - type 1	Direct Detection	Real Time PCR	Diagnostic		21 days	CSF
Herpes simplex virus - type 2	Direct Detection	Real Time PCR	Diagnostic		21 days	CSF
Herpes virus (HSV)	Isolation	Cell Culture	Diagnostic		14 days	oral swab or cells from base of lesion
Human Herpes Virus 6 (HHV6)	Direct Detection	Real Time PCR	Diagnostic		21 days	CSF
			Diagnostic			
Human metapneumovirus (hMPV)	Direct Detection	Real Time PCR	Diagnostic		14 days	respiratory
Human Immunodeficiency Virus (HIV-1/HIV-2 PLUS O Combi)	IgG & IgM	EIA	Diagnostic		14 days	serum
Human Immunodeficiency Virus type 1 (HIV-1)	IgG	FA	Diagnostic		14 days	serum
Human Immunodeficiency Virus type 1 (HIV-1)	IgG	Western blot	Diagnostic		14 days	serum
Human Immunodeficiency Virus type 2 (HIV-2)	IgG	EIA	Diagnostic		21 days	serum
Human Immunodeficiency Virus type 2 (HIV-2)	IgG	FA	Diagnostic		21 days	serum
Human Immunodeficiency Virus type 2 (HIV-2)	IgG	Western blot	Diagnostic		21 days	serum
			Diagnostic			
Human T Cell Lymphotropic Virus (HTLV) I & II	IgG	EIA	Diagnostic		14 days	serum
Human T Cell Lymphotropic Virus (HTLV) I & II	IgG	IFA	Diagnostic		14 days	serum
Human T Cell Lymphotropic Virus (HTLV) I & II	IgG	Western blot	Diagnostic		31 days	serum
Human T Cell Lymphotropic Virus (HTLV) I & II	IgG	RIPA	Diagnostic		90 days	serum
Influenza A	IgG	EIA	Diagnostic		14 days	acute and convalescent sera
Influenza A	Isolation	Cell Culture	Diagnostic		21 days	respiratory

VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 11/18/2008	ASSAY	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT	SAMPLE
Influenza A	Strain Typing	HI	Non-diagnostic		120 days	Cell Culture Isolate
Influenza A	Direct Detection	Real Time PCR	Diagnostic		14 days	respiratory
Influenza A subtyping for H1, H3, H5	Direct Detection	Real Time PCR	Non-diagnostic		14 days	respiratory
Influenza B	IgG	EIA	Diagnostic		14 days	acute and convalescent sera
Influenza B	Isolation	Cell Culture	Diagnostic		21 days	respiratory
Influenza B	Strain Typing	HI	Non-diagnostic		120 days	Cell Culture Isolate
Influenza B	Direct Detection	Real Time PCR	Diagnostic		14 days	respiratory
Isolate for Identification	Isolation	Cell Culture	Diagnostic		28-120 days	Cell culture isolate
Lymphocytic choriomeningitis (LCM)	IgG	IFA	Diagnostic		14 days	serum
Measles	IgG & IgM	EIA	Diagnostic	Case history required	7 days	serum
Measles	IgG & IgM	IFA	Diagnostic		7 days	serum
Measles	Isolation	Cell Culture	Diagnostic		28 days	Respiratory
Measles	Direct Detection	Real time PCR	Diagnostic		14 days	Respiratory
Milker's nodules (Orf, Cowpox)	IgG & IgM	IFA	Diagnostic		14 days	serum
Milker's nodules (Orf, Cowpox)	Direct Detection	DFA	Diagnostic		5 days	vesicular swab / scab
Mumps	IgG	EIA	Diagnostic		14 days	serum
Mumps	IgG & IgM	IFA	Diagnostic		14 days	serum
Mumps	Isolation	Cell Culture	Diagnostic		28 days	Bucal swab
Mumps	Direct Detection	Real time PCR	Diagnostic		14 days	Respiratory
<i>Mycoplasma pneumoniae</i>	IgG & IgM	EIA	Diagnostic		14 days	serum
<i>Mycoplasma pneumoniae</i>	Direct Detection	Real Time PCR	Diagnostic		28 days	respiratory or CSF
Norovirus (includes Norwalk virus)	Direct Detection	Real Time PCR	Non-diagnostic	stools from outbreaks only	14 days	4-10 stools per outbreak
Norovirus strain typing	Direct Detection	PCR then sequencing	Non-diagnostic	isolates from outbreaks	120 days	RNA positive extracts
Parainfluenza type 1	Direct Detection	Real Time PCR	Diagnostic		14 days	respiratory

VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 11/18/2008	ASSAY	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT	SAMPLE
Parainfluenza type 2	Direct Detection	Real Time PCR	Diagnostic		28 days	respiratory
Parainfluenza type 3	Direct Detection	Real Time PCR	Diagnostic		14 days	respiratory
Parainfluenza type 4	Direct Detection	Real Time. PCR	Diagnostic		14 days	respiratory
Rabies (suspected human case)	IgG and IgM	IFA	Diagnostic	requires encephalitis case history and prior consultation	3 days	serum
Rabies (suspected human case)	Direct Detection	IFA	Diagnostic		3 days	various - call for medical consultation
Rabies (immune status)	IgG	RFFIT	Diagnostic	limited to PH staff	120 days	serum
Rabies (animal)	Direct Detection	DFA	Diagnostic		3 days	cross section of brain stem and cerebellum
Respiratory syncytial (RSV)	Direct Detection	Real Time PCR	Diagnostic		14 days	respiratory
Rhinoviruses	Isolation	Cell Culture	Diagnostic		28 days	respiratory
Rhinoviruses	Direct Detection	Real Time PCR then gel electrophor esis	Diagnostic		28 days	respiratory
Rickettsia <i>typhi</i> (typhus)	IgG & IgM	IFA	Diagnostic	Case history required	14 days	serum
Rocky Mountain spotted fever (RMSF)	IgG & IgM	IFA	Diagnostic		14 days	serum
Rubella (German measles)	IgG & IgM	EIA	Diagnostic		14 days	serum
Rubella (German measles)	Isolation	Cell Culture	Diagnostic		28 days	throat swab or urine
Sin Nombre Virus Hantavirus Pulmonary Syndrome	IgG & IgM	EIA	Diagnostic	case history required	14 days	serum
Vaccinia (vaccine strain)	IgG & IgM	IFA	Diagnostic	prior consultation required	14 days	serum
Varicella-zoster (Herpes Zoster)	IgG & IgM	EIA	Diagnostic		14 days	serum
Varicella-zoster (Herpes Zoster)	Isolation	Cell Cultue	Diagnostic		28 days	lesion swab

VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 11/18/2008	ASSAY	METHOD	STATUS	SPECIAL REQUIREMENTS	TAT	SAMPLE
Varicella-zoster (Herpes Zoster)	Direct Detection	DFA	Diagnostic		3 days	basal cells from lesion
Varicella-zoster (Herpes Zoster)	Direct Detection	Real Time PCR	Diagnostic		14 days	CSF
The use of LRN PCR assays are restricted to the investigation of possible BT events or other Public Health emergencies						
Avian Influenza (H5N1)	Direct Detection	Real Time PCR	Non-routine	prior consultation required	2 days	scabs / lesion swab
Non-Variola Orthopox	Direct Detection	Real Time PCR	Non-routine	prior consultation required	2 days	scabs / lesion swab
Q fever	Direct Detection	Real Time PCR	Non-routine	prior consultation required	2 days	blood
Vaccinia (vaccine strain)	Direct Detection	Real Time PCR	Non-routine	prior consultation required	2 days	scabs / lesion swab
Variola (smallpox)	Direct Detection	Real Time PCR	Non-routine	prior consultation required	2 days	scabs / lesion swab
Varicella Zoster (Herpes Zoster)	Direct Detection	Real Time PCR	Non-routine	prior consultation required	2 days	scabs / lesion swab

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) Mumpsvirus Herpesvirus LCM 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 Herpesvirus Post-infectious mumps, measles, rubella, influenza
Rabies	Acute encephalitis; similar to aseptic meningitis plus; onset begins with sense of apprehension, indefinite sensory changes; disease progresses to paresis or paralysis; hydrophobia, delirium, convulsions; respiratory paralysis.	Rabiesvirus
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.

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
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
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, Chlamydia 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		
Measles	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

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Roseola infantum	Leucopenia, sometimes marked. Typically childhood disease in infants less than 4 years. High fever for 3 days then transient generalized rubella-like rash as fever falls.	Human Herpes Virus type 6 (HHV 6). (A newly recognized herpes virus, HHV 7 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
Rocky Mt. Spotted Fever (RMSF)	Fever, headache, myalgias, malaise, petechial rash, tick bite or exposure to ticks	Rickettsia rickettsi
Ehrlichiosis (monocytic) Human Granulocytic Ehrlichiosis (HGE)	similar to Rocky Mt. Spotted Fever and Lyme Disease but usually without any rash. Transmitted by tick.	Ehrlichia chaffeensis (tropism for leukocytes (monocytes, lymphocytes and neutrophils); Ehrlichia _____ (HGE) (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, usual venereal. Generalized spread may occur.	Herpes virus type I Herpes virus 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)

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Varicella-Zoster (Herpes-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)
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.	Herpesvirus 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.
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		
Rubella syndrome	Varied defects including deafness, eye-defects, microphthalmia, 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

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
Herpes simplex	Congenital defects when fetus is infected; often fatal generalized infection or permanent 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
Section #6 - PERINATAL INFECTIONS		
AIDS	Pediatric AIDS can be transmitted in utero, intrapartum or via breast milk. HIV laboratory results can be confusing to interpret due to 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 (infectious hepatitis)	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 (serum hepatitis)	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
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.

DISEASE OR SYNDROME	TYPICAL CLINICAL FEATURES	ETIOLOGIC AGENTS
<p>Adult T-cell Leukemia</p> <p>HTLV associated myelopathy (HAM also Tropical Spastic Paraparesis)</p>	<p>Lymphadenopathy, hepatomegaly, splenomegaly, cutaneous lesions without severe itching or excoriation, some immune deficiencies.</p> <p>slowly progressive lower extremity weakness and spasticity with variable sensory changes and spinal cord demyelination.</p>	<p>HTLV-I</p>
<p>HTLV-II</p>	<p>not yet linked with a specific clinical illness but antibodies are common in IV drug abusers.</p>	<p>HTLV-II</p>
<p>Acquired Immunodeficiency Syndrome (AIDS),</p> <p>Aids Related Complex (ARC)</p>	<p>Acute Syndrome: fever, malaise, myalgia, arthralgia, headache, macular rash and lymphadenopathy.</p> <p>ARC syndrome: persistent generalized lymphadenopathy, oral candidiasis, fever and weight loss.</p> <p>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.</p>	<p>Human Immunodeficiency Virus (HIV) types I and II</p>
<p>Section #9 - GASTROINTESTINAL</p>		
<p>Epidemic Viral Gastroenteritis</p>	<p>Usually a self-limited mild disease with nausea, vomiting, diarrhea, abdominal pain, myalgia, headache, malaise, low grade fever . Symptoms usually last 24-48 hrs.</p>	<p>Small Round Structured Virus (SRSV) Grouped into Astro and Caliciviruses based on EM morphology. (Norwalk virus is a member of the calicivirus group)</p>
<p>Sporadic Viral Gastroenteritis (Winter Vomiting Disease)</p>	<p>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 10¹² particles/ml of stool.</p>	<p>Rotavirus</p>

