



VIRAL AND RICKETTSIAL DISEASE LABORATORY

GUIDELINES FOR LABORATORY SERVICES

UPDATED: 1/27/2012

| Version | Date | Author/Editor | Laboratory Director |
|---|-------------|----------------------|----------------------------|
| 5.4.3 | 01/27/2012 | D Cottam | Dr. Hanson |
| <i>Brief Description of Changes:</i> | | | |
| <ol style="list-style-type: none"> 1. Updated Table of VRDL Assays to remove the following analytes from Diagnostic tests offered: 2. Housekeeping – minor corrections to grammar and formatting 3. Clarified that mumps serology is most useful when acute and convalescent samples can be compared or when the patient has not been immunized. In a recent mumps outbreak we had several cases of mumps (confirmed by PCR) that did not show any detectable mumps IgM antibody (page 22) 4. Added Statement - a buccal swab is the specimen of choice for mumps isolation attempts and/or PCR testing. (see page 12 – Note #7) 5. We have documented that in several laboratory confirmed cases of mumps (by PCR), no mumps IgM antibody could be detected by IFA. Thus it is not very meaningful to test for mumps IgM unless the patient has not be immunized. (see page 22) | | | |

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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 (IFA) or antigen detection (DFA)) |
| 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 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. Dr. Sharon Messenger assumed the leadership on February 1, 2011 and is our current acting acting Branch Chief while Dr. Carl Hanson is our acting Laboratory Director.

Mission Statement

The Viral and Rickettsial Disease Laboratory 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, 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 Viral Immunoserology and Molecular Diagnostic Section** performs antibody testing and nucleic acid detection for over 20 different infectious diseases such as influenza, herpes simplex, varicella zoster virus, measles, mumps, rubella and arboviruses [e.g. West Nile Virus (WNV)].
- **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, influenza and norovirus.
- **The Epidemiology Support Section** provides testing services on all aspects of the diagnosis, treatment, virology, immunology, and epidemiology of human immunodeficiency virus 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, sample collection and shipment and interpretation of test results. Our clients include other branches of CDPH, local public health laboratories, clinical laboratories, and physicians throughout the state. This section coordinates several statewide projects including the California Encephalitis Project, West Nile Virus Project, Sentinel Influenza Physician Surveillance Project, 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 www.CAPHLD.org

There are currently 38 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 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 | 530-642-8531 |
| Fresno | 1221 Fulton Mall, Fresno, CA 93721 | 559-445-7008 | 559-445-7080 |
| 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 | 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 |
| Marin | 920 Grand Avenue, San Rafael, CA 94901 | 415-499-6849 | 415-507-2986 |
| 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 | 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 |
| Pasadena | 1845 North. Fair Oaks Ave Suite P310, Pasadena, CA 91103 | 626-744-6011 | 626-744-6126 |
| Placer | 11475 C Avenue, Auburn, CA 95603 | 530-889-7210 | 530-889-7209 |

| JURISDICTION | MAILING ADDRESS | PHONE | FAX |
|-----------------|--|--------------|--------------|
| 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 |
| San Bernardino | 799 East Rialto Ave., San Bernardino, CA 92415 | 909-383-3000 | 909-383-3094 |
| 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. 2 nd 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 | 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 |
| Sutter | 1445 Veterans Memorial Circle, Yuba City, CA 95993 | 530-822-7225 | 530-822-7074 |
| 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 | 805-981-5130 |

VRDL Contact Information

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

VRDL main phone # (510) 307-8575; main fax # (510) 307-8599

| Section / Special Project | Section Supervisors / Project Coordinators |
|--|--|
| Medical and Epidemiology Liaison Section Local Assistance | David Cottam (510) 307-8585 David.Cottam@cdph.ca.gov Ray Sante, Anthony Moore, Chris Anderson |
| CA Encephalitis Project | Heather Sheriff (510) 307-8607 Heather.Sheriff@cdph.ca.gov |
| West Nile Virus Project | Maria Salas and Katharine King (510) 307-8606 (510) 307-8562 Maria.Salas@cdph.ca.gov Katharine.King@cdph.ca.gov |
| Influenza Sentinel Providers | Katharine King and Christopher Anderson (510) 307-8562 (510) 307-8585 Katharine.King@cdph.ca.gov Christopher.Anderson@cdph.ca.gov |

Other Contact Information

California Department of Public Health - Microbiology Disease Laboratory Branch (MDL) (510) 412-3700

| | |
|--|----------------|
| California Department of Public Health Infectious Disease Branch | (510) 620-3434 |
| Veterinary Public Health Section | (916) 552-9740 |
| Vector Borne Disease Section | (916) 552-9730 |
| Immunization Branch | (510) 620-3737 |
| Communicable Disease Emergency Response (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.
- 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 a 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 with the approval of the Laboratory Chief or Medical Officer.

| 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:

- Patient Name or Patient Identification Number (must also be written on the sample container)
- Date of Birth
- Date of Onset (if applicable)
- 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 assays Plasma is acceptable for HIV and HTLV 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 |
| Respiratory Samples Nasopharyngeal, throat & nasal swab, endotracheal aspirates, bronchial washing See note #7 | 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 | 4°C / cold pack | -70°C / Dry Ice |
| Buccal swabs for suspected mumps See note #7 | 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 | 4°C / cold pack | -70°C / Dry Ice |
| Stool for isolation or direct detection (Polymerase Chain Reaction) See note #1 | ASAP – not later than 7 days after onset | 2-4 grams | None | 4°C / cold pack | 4°C / cold pack |
| Rectal swabs for isolation or direct detection (PCR) See note #2 | 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 |
| 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 pack | 4°C / cold pack |
| Urine see note #3 | ASAP - within 7 days | 10 – 40 ml | See note #3 | See note #3 | See note #3 |
| 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. 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. However, measles can frequently be detected in urine samples by PCR from suspected measles cases and we continue to recommend that they be collected and submitted for testing. 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 – 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 (taken ASAP but within 7 days of the date of onset)- acute specimens of particularly high public health significance for which reliable IgM tests exist are tested as soon as possible. Testing a single acute blood for other requests is generally not useful and a convalescent (collected 14-21 days after the date of onset) specimen 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).

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 can be useful as in the case for herpesvirus where a negative antibody result 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.

- A CSF specimen (1) taken within a few days of the date of onset and shipped promptly at 4°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 and will be routed for isolation attempts if above conditions are met.

- 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 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 provided that the CSF is a validated specimen type for the respective assay.

Note #7 – Notes on swabs for virus isolation and PCR testing

- Dacron swabs with plastic sticks should be used to collect samples for virus isolation. Cotton or cotton alginate swabs and wooden handles contain oils which are inhibitory to viral growth.
- 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 attempts.
- A buccal swab is the specimen of choice for mumps isolation attempts and/or PCR testing.
- Stools are much superior to rectal swab for virus isolation and/or PCR. 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 include:

- Avian (or other pandemic) Influenza
- California Encephalitis Project
- Culture for Identification
- Gastroenteritis Outbreaks – Suspected Norovirus
- Gastroenteritis Outbreaks – RNA extracts from positive stools
- Hantavirus – Human Pulmonary Syndrome (HPS)
- Influenza and other Respiratory Illness
- 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. The most up-to-date versions of these forms are available in PDF format on the 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)

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
- 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.

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.

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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
(list continues on the following page)
- 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>

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 under Special Instructions “**RING BELL AT GATE FOR ADMITTANCE**”.
- 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, 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 or faxed 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 or by fax.

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 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 –

Members of the Group B arboviruses (including Flaviviruses such as St. Louis encephalitis, 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.

Members of the Group A arbovirus (including Togaviruses such as Western equine encephalitis and Chikungunya) may produce antibodies that cross-react with one another.

Arboviruses endemic in California

EIA is performed for Western equine encephalitis (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:

- The Viral and Rickettsial Disease Laboratory [VRDL] will test serum samples for WNV. VRDL recommends residual CSF also be submitted for WNV testing when a lumbar puncture has been performed for further testing at CDC if necessary
- When previously untested serum is received, enzyme immunoassay (EIA) is done on serum (CSF is stored in case additional confirmatory testing is needed)
- Immunofluorescence assay (IFA) may be done as an adjunct test on serum
- 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.
- Please consult with VRDL for guidance any time WNV is strongly suspected, regardless of previous test results.
- Plaque reduction neutralization testing (PRNT) is done to resolve indeterminate results, or by request (Note: At VRDL, PRNT is not currently validated for diagnostic purposes; these results are to be used for surveillance purposes only)
- Enterovirus PCR may also be done on CSF specimens on a seasonal basis, depending on the availability of resources at VRDL - Call 510-307-8606 to find out whether the most current algorithm includes enterovirus PCR

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). Samples are tested for CHIK antibody by Western blot. Samples can also be forwarded to CDC for additional testing (serology, PCR and virus isolation).

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 attempt strain typing on very acute serum in cases of special public health interest.

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.

St. Louis Encephalitis (SLE) and Yellow Fever are not offered as a routine service and VRDL and requests for these two viruses will be sent to CDC for specific testing

Encephalitis

The VRDL California Encephalitis Project attempts 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. For more information about either project, please visit the Project's website (www.ceip.us) or call (510) 307-8608.

As the CEP panel includes testing for several agents (see table), the turnaround time for completion of core testing is 8 weeks. If additional tests are warranted (based on exposure, clinical symptoms or laboratory data) or specimens are sent off-site for testing, this turnaround time may exceed 8 weeks.

CEP Core Testing as of 2011

| Specimen Type | Agent | Test Type |
|----------------------------|--|-----------|
| CSF | HSV-1 | PCR |
| | HSV-2 | PCR |
| | VZV | PCR |
| | HHV-6 | PCR |
| | Enterovirus | PCR |
| | Measles, HSV, VZV* | Serology |
| Acute & Convalescent serum | West Nile Virus* | Serology |
| | Epstein-Barr virus* | Serology |
| | <i>Mycoplasma pneumoniae</i> (Myco IgM testing is not currently available) | Serology |
| | Influenza A and B* | Serology |
| | Adenovirus | Serology |
| | HSV* | Serology |
| | Measles* | Serology |
| VZV* | Serology | |
| Respiratory sample | Enterovirus | PCR |
| | <i>Mycoplasma pneumoniae</i> | PCR |
| | General Viral Isolation* | Culture |

| | |
|--|-----|
| Respiratory panel*: Influenza A and B, Adenovirus, Human Metapneumovirus, RSV type A, Parainfluenza Types 1-4. | PCR |
|--|-----|

Core testing in **bold**.

*Testing done as needed based on exposure, seasonality or clinical presentation

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 outbreaks 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 form 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 while vomitus may contain high norovirus titers, our PCR assay 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 is unclear and 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 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 will not be tested since neither a positive nor negative result will be meaningful. 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 4°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 should never be frozen.

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.

Norovirus strain typing (PCR positives from the Local HD)

Local health departments 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 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 either the Centers for Disease Control and Prevention or a reference laboratory at the University of New Mexico.

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.
- Varicella for special situations of unusual public health importance (such as a hospital outbreak where an immediate answer is necessary for staff or patient management). For suspected cases of varicella – DFA on smears prepared from lesion basal cells provides rapid laboratory results
- 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. This test is also performed at 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.

Infectious Mononucleosis

The VRDL offers serologic testing for Epstein Barr Virus (EBV), the causative agent for infectious mononucleosis. The combination of testing for VCA IgG, VCA IgM and EBNA antibodies usually provides a good indicator of when a patient was infected.

Rabies virus

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

Rabies (Animal) Requests for animal testing should be referred to the local health departments. Local health departments may request animal brain testing if there has been significant human exposure.

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.

Retrovirus Samples

HIV Serology

- Specimens sent to our laboratory for HIV testing are usually screened by Enzyme Immunoassay (EIA) and Immunofluorescence Assay (IFA)
- 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.
- Dried blood spots are no longer accepted for HIV serology.

HTLV Serology

- Specimens are usually 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 – no test results agree
- Unsatisfactory - sample was nonspecific or inappropriate for testing

Western blot interpretations require at least the following bands:

| | |
|---------------|--|
| Positive | p19 and/or p24 plus p21e bands |
| Indeterminate | p21e band only |
| Negative | p21e band is absent. Regardless of core bands (p19 and/or p24) |

Rickettsial Agents

Specimen Collection for Rickettsial Testing

Although it is technically feasible, specific rapid laboratory confirmation of rickettsial diseases is rarely done. Therefore, treatment decisions should be based on epidemiologic and clinical clues, and should never be delayed while waiting for confirmation by laboratory results. The majority of patients demonstrate increased IgM or IgG titers by the second week of the illness (patients infected with certain imported rickettsiae might not demonstrate increased titers until 4 weeks after illness onset). However, patients might lack diagnostic IgG and IgM antibody titers in the first 7 days of illness, a period when the majority of patients initially seek medical care and laboratory testing is performed.

The indirect immunofluorescence assay (IFA) is generally considered the reference standard and is the test currently used by VRDL to test for *Rickettsia rickettsii* (Rocky Mountain Spotted Fever), *R. typhi* (murine typhus), *Coxiella burnettii* (Q fever), [Anaplasma phagocytophilum](#) ([human granulocytic anaplasmosis](#)) and *Ehrlichia chafeensis* ([human monocytic ehrlichiosis](#)). Paired serum specimens taken early (i.e., acute) and later (i.e., convalescent) in the disease course represent the preferred specimens for evaluation. Typically, these specimens should be taken at least 2--3 weeks apart to examine for a four-fold or greater increase in antibody titer. IgG antibodies are more specific and reliable since other bacterial infections can also cause elevations in rickettsial IgM antibody titers; therefore samples will be screened for IgG antibody and if positive, are reflexed for IgM testing.

For rickettsial agents, the value of testing two sequential sera together to show a rising antibody level is very important in confirming acute infection with rickettsial agents because antibody titers may persist in some individuals for years after the original exposure to any of a number of rickettsial agents. Cross-reactivity among rickettsial antigens is common.

Minimum specimen requirement:

- Acute and convalescent sera (5-10 cc) should be collected in a red top or tiger top tube.

Additional desired specimens, as available:

- Currently VRDL is developing testing for better confirmation of current IFA results as well as investigation of 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 future testing in addition to IFA will include isolation, western blot and referral to CDC for the same tests as well as PCR and immunohistochemistry of tissue. To assist with surveillance studies, for all cases where ANY rickettsial infection is suspected, the following samples should also be requested (as available):
 - 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).

Note - Eschar should be sent dry or with a little saline as possible since very little virus is usually present and adding saline dilutes the sample and makes it harder to detect any virus present.

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

Submitters are reminded that serologic testing remains the primary laboratory method, in conjunction with assessment of the relevant epidemiologic and clinical history, for ruling in or ruling out acute measles infection.

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.

The most reliable sample for serology testing is blood collected in red top tubes (5 cc is ideal but 1-2 cc is acceptable) for optimal results.

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 and the serum removed from the clot.
- 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 strain typing.

Measles Isolation – The availability of a sensitive cell lines (Vero or Slam) for measles isolation has greatly increased our ability to isolate measles virus. 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 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. Otherwise 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 – Sometimes measles can still be detected and/or recovered from a urine sample after it is no longer present in the throat. If possible, 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 PCR- For cases of suspected measles, please collect a respiratory swab (nasopharyngeal, nasal or oropharyngeal) and urine in addition to sera. Measles virus can be detected in respiratory epithelium of the nasopharynx tissue early in the infection (0 – 7 days from rash onset), so the positive predictive value of the real-time PCR for measles is high. However, a negative result cannot be used to “rule out” measles. Measles virus can frequently be detected in the urine later in the infection (up to 10 days – sometimes longer) when it can no longer be detected in respiratory samples. The PCR results should always be interpreted in conjunction with serologic testing for IgM and IgG and thorough assessment of the relevant clinical and epidemiologic risk factors.

- **Submitters are reminded that serologic testing remains a primary laboratory method and we encourage submitting samples for both serological and direct detection (PCR) whenever possible.**

Mumps

Mumps Serology - VRDL currently performs an EIA to measure mumps IgG antibodies and an IFA assay to measure mumps IgM and IgG antibodies but results must be interpreted with caution since false positive or non-specific reactions are known to occur. We have documented that in several laboratory confirmed cases of mumps (by PCR), no mumps IgM antibody could be detected by IFA. Thus it is not very meaningful to test for mumps IgM unless the patient has not been immunized. The gold standard for serological testing remains the demonstration of a significant change (4 fold rise) in titer between the acute and convalescent samples.

Mumps Isolation - Buccal swabs collected as soon as possible but not later than 9 days of onset of parotitis. Place buccal swabs in 2-3 ml of VTM. Samples which can be received by the VRDL within 72 hours should be stored and shipped at 4°C. Otherwise the buccal swabs should be stored and shipped at -70°C or colder. Urine samples are not routinely recommended.

Mumps Direct Detection by PCR – The VRDL has completed the validation of a mumps PCR assay. Collection, storage and transport instructions for the buccal swabs are the same as for mumps isolation above. Please also submit a serum as well. Urine samples are not recommended.

Rubella

Rubella Serological testing VRDL currently 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. The gold standard for serological assays is the detection of a significant change (4 fold rise) between the acute and convalescent serum samples.

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.

- Rubella PCR- The VRDL has completed the validation of a rubella PCR assay. For cases of suspected rubella, please collect a respiratory swab (nasopharyngeal, nasal or oropharyngeal) and urine in addition to sera. The PCR results should always be interpreted in conjunction with a thorough assessment of the relevant clinical and epidemiologic risk factors.

Polio -

Polio Isolation and PCR - Poliovirus can be grown in cell culture and is detectable using our enterovirus PCR assay.

No routine service for checking immunity status.

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 Serology – Significant rise (4 fold rise) in varicella IgG antibody levels between acute and convalescent serum is the gold standard for varicella serological testing. Varicella IgM by EIA is also offered.

Varicella Isolation and Direct Detection – The optimal test for cases of suspected chicken pox or shingles is the direct detection test. Sample collection is best accomplished by removing the scab of the lesion, discarding any pus and then collecting 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 (for direct detection) and then place the swab into 1-2 ml of VTM (for isolation attempts). 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. Varicella PCR is described on the following page.

Varicella PCR. The VRDL has completed the validation of a PCR assay for detection of VZV nucleic acid in the vesicle swabs (to collect basal cells) scabs or lesions. Please place several scabs in a clean container and send along with the smear on the microscope slide and the swab that was placed in VTM for isolation attempts.

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

Appendix A Table of VRDL Assays – Sorted by Agent

Updated: January 27, 2012

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.

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. NOTE: See page 16 for TAT for samples submitted to the Encephalitis Project.

| VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 2/2/2011 | ASSAY | METHOD | STATUS | SPECIAL REQUIREMENTS | TAT | SAMPLE |
|--|----------------|--------|----------------|----------------------------|------------|--------|
| Arboviruses | | | | | | |
| Chikungunya | Antibody | Blot | Diagnostic | Travel history required | 30-60 days | Serum |
| Chikungunya | IgG & IgM | IFA | Diagnostic | Travel history required | 14 days | Serum |
| Dengue (does not distinguish type) | IgG | EIA | Diagnostic | | 30 days | Serum |
| Dengue (does not distinguish type) | IgG & IgM | IFA | Diagnostic | Travel history required | 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 | | 14 days | Serum |
| West Nile Virus (WNV) | IgG & IgM | IFA | Diagnostic | Case history required | 14 days | Serum |
| West Nile Virus (WNV) | Antibody | Blot | Non-diagnostic | | 14 days | Serum |
| West Nile Virus (WNV) | Neutralization | PRNT | Non-diagnostic | | 28 days | Serum |
| Western Equine Encephalitis (WEE) | IgG | EIA | Diagnostic | Case history required | 14 days | Serum |
| Western Equine Encephalitis (WEE) | Neutralization | PRNT | Non-diagnostic | | 28 days | Serum |

| VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 2/2/2011 | ASSAY | METHOD | STATUS | SPECIAL REQUIREMENTS | TAT | SAMPLE |
|--|----------|--------|----------------|-------------------------|---------|--------|
| Western Equine Encephalitis (WEE) | Antibody | Blot | Non-diagnostic | | 14 days | Serum |

Other Viruses and Rickettsial Agents in alphabetical order.

| | | | | | | |
|---|---------------------|---------------|----------------|--------------------------------|---------|--|
| Adenovirus | IgG | EIA | Diagnostic | | 14 days | Serum |
| Adenovirus | IgG & IgM | IFA | Non-diagnostic | | | Serum |
| Adenovirus | Isolation | Cell culture | Diagnostic | | 28 days | Respiratory |
| Adenovirus | Direct Detection | Real Time PCR | Diagnostic | | 14 days | Respiratory |
| | | | | | | |
| <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 | | 28 days | Respiratory or urine |
| | | | | | | |
| <i>Ehrlichia chaffeensis</i> | IgG & IgM | IFA | Diagnostic | Case history required | 14 days | Serum |
| <i>Anaplasma phagocytophilia</i> (HGE) | IgG & IgM | IFA | Diagnostic | | 14 days | Serum |
| | | | | | | |
| Enterovirus | Isolation | Cell Culture | Diagnostic | | 28 days | Respiratory or Fecal |
| Enterovirus | IgM | EIA | Non-diagnostic | | 14 days | Serum or CSF |
| Enterovirus | Direct Detection | Real Time PCR | Diagnostic | | 14 days | CSF |
| Enterovirus | IgG | Serum Neut | 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 |
| 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 |

| VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 2/2/2011 | ASSAY | METHOD | STATUS | SPECIAL REQUIREMENTS | TAT | SAMPLE |
|--|---------------------|---------------|----------------|---------------------------------|------------|--|
| Herpes simplex 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 | | 21 days | Serum or plasma |
| Human Immunodeficiency Virus type 1 (HIV-1) | IgG | FA | Diagnostic | | 21 days | Serum or plasma |
| Human Immunodeficiency Virus type 1 (HIV-1) | IgG | Western blot | Diagnostic | | 21 days | Serum or plasma |
| Human Immunodeficiency Virus type 2 (HIV-2) | IgG | EIA | Diagnostic | | 21 days | Serum or plasma |
| Human Immunodeficiency Virus type 2 (HIV-2) | IgG | FA | Diagnostic | | 21 days | Serum or plasma |
| Human Immunodeficiency Virus type 2 (HIV-2) | IgG | Western blot | Diagnostic | | 21 days | Serum or plasma |
| | | | | | | |
| Human T Cell Lymphotropic Virus (HTLV) I & II | IgG | EIA | Diagnostic | | 14 days | Serum or plasma |
| Human T Cell Lymphotropic Virus (HTLV) I & II | IgG | IFA | Diagnostic | | 14 days | Serum or plasma |
| Human T Cell Lymphotropic Virus (HTLV) I & II | IgG | Western blot | Diagnostic | | 31 days | Serum or plasma |
| Human T Cell Lymphotropic Virus (HTLV) I & II | IgG | RIPA | Diagnostic | | 90 days | Serum or plasma |
| | | | | | | |
| Influenza A | IgG | EIA | Diagnostic | | 14 days | Acute and Convalescent sera |
| Influenza A | Isolation | Cell Culture | Diagnostic | | 21 days | Respiratory |
| 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 and Pandemic Influenza A (H1) 2009 | Direct Detection | Real Time PCR | Diagnostic | | 14 days | Respiratory |
| Influenza A B screening | Direct Detection | Real Time PCR | Diagnostic | | 14 days | Respiratory |
| Influenza B | IgG | EIA | Diagnostic | | 14 days | Acute and Convalescent sera |
| Influenza B | Isolation | Cell Culture | Diagnostic | | 21 days | Respiratory |

| VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 2/2/2011 | ASSAY | METHOD | STATUS | SPECIAL REQUIREMENTS | TAT | SAMPLE |
|--|------------------|---------------------|----------------|---|-------------|---|
| 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 (rubeola) | IgG & IgM | EIA | Diagnostic | For suspected cases, please contact the local public health department and complete a case history form | 7 days | Serum |
| Measles (rubeola) | IgG & IgM | IFA | Diagnostic | | 7 days | Serum |
| Measles (rubeola) | Isolation | Cell Culture | Diagnostic | | 28 days | Respiratory |
| Measles (rubeola) | Direct Detection | Real time PCR | Diagnostic | | 7 days | Respiratory and Urine |
| Orf, Cowpox | IgG & IgM | IFA | Diagnostic | | 14 days | Serum |
| Orf, Cowpox | Direct Detection | 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 historyform | 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 | Bucal swab |
| <i>Mycoplasma pneumoniae</i> | IgG | 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 | Nucleic acid extracts from outbreaks | 120 days | RNA positive extracts |
| Parainfluenza types 1 - 4 | Isolation | Cell Culture | Diagnostic | | 14 days | Respiratory |
| Parainfluenza types 1 - 4 | Direct Detection | Real Time PCR | Diagnostic | | 14 days | Respiratory |
| Rabies (suspected human case) | IgG and IgM | IFA | Diagnostic | Suspected Human Rabies requires encephalitis case history and prior consultation | 3 days | Serum |
| Rabies (suspected human case) | Direct Detection | DFA | Diagnostic | | 3 days | Various - Call for medical consultation |

| VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 2/2/2011 | ASSAY | METHOD | STATUS | SPECIAL REQUIREMENTS | TAT | SAMPLE |
|--|---------------------|---------------------------------|------------|--------------------------------|----------|--|
| 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 | PCR then gel electrophoresis | Diagnostic | | 28 days | Respiratory |
| <i>Rickettsia typhi</i> (typhus) | IgG & IgM | IFA | Diagnostic | Case history requested | 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 |
| Rubella (German measles) | Direct Detection | PCR | Diagnostic | | 14 days | Respiratory |
| Sin Nombre Virus Hantavirus Pulmonary Syndrome | IgG & IgM | EIA | Diagnostic | Case history required | 14 days | Serum |
| Vaccinia (vaccine strain) | IgG | 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 |
| Varicella-zoster (Herpes Zoster) | Direct Detection | DFA | Diagnostic | | 3 days | Lesion swab and/or Basal Cells from lesion |
| Varicella-zoster (Herpes Zoster) | Direct Detection | Real Time PCR | Diagnostic | | 14 days | Lesion swab and/or Basal Cells from lesion CSF |

| VIRAL AND/OR RICKETTSIAL AGENTS UPDATED: 2/2/2011 | ASSAY | METHOD | STATUS | SPECIAL REQUIREMENTS | TAT | SAMPLE |
|--|---------------------|---------------|-------------|--------------------------------|--------|-----------------------------------|
| 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 | Upper and lower respiratory |
| Non-Variola Orthopox Note #1 | 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 |
| Varicella Zoster (Herpes Zoster) | Direct Detection | Real Time PCR | Non-routine | Prior consultation required | 2 days | Scabs / Lesion swab |
| <p>Note #1 Acceptable samples 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.</p> | | | | | | |

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. |
| 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 |

| DISEASE OR SYNDROME | TYPICAL CLINICAL FEATURES | ETIOLOGIC AGENTS |
|---|--|---|
| 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 |
| 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 (HHV 6). (A newly recognized herpes virus, HHV 7 may also be a causative agent.) |

| DISEASE OR SYNDROME | TYPICAL CLINICAL FEATURES | ETIOLOGIC AGENTS |
|--|--|--|
| 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 however rash may (or may not) be associated. Transmitted by tick. | Ehrlichia chaffeensis (tropism for leukocytes (monocytes, lymphocytes and neutrophils); <i>Anaplasma phagocytophilum</i> (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) |
| 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) |

| DISEASE OR SYNDROME | TYPICAL CLINICAL FEATURES | ETIOLOGIC AGENTS |
|---|---|--|
| 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 |
| 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 |

| DISEASE OR SYNDROME | TYPICAL CLINICAL FEATURES | ETIOLOGIC AGENTS |
|--|---|---|
| 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 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 (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. |
| Adult T-cell Leukemia | Lymphadenopathy, hepatomegaly, splenomegaly, cutaneous lesions without severe itching or excoriation, some immune deficiencies. | HTLV-I |
| HTLV associated myelopathy (HAM also Tropical Spastic Paraparesis) | slowly progressive lower extremity weakness and spasticity with variable sensory changes and spinal cord demyelination. | |
| HTLV-II | not yet linked with a specific clinical illness but antibodies are common in IV drug abusers. | HTLV-II |

| DISEASE OR SYNDROME | TYPICAL CLINICAL FEATURES | ETIOLOGIC AGENTS |
|---|---|--|
| <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> |

