Viral and Rickettsial Disease Laboratory

Annual Report

2007 - 2008

California Department of Public Health

Center for Infectious Diseases

Division of Communicable Disease Control

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Our many partners and collaborators in the Local Health Departments, particularly the Public Health Laboratories;

Our many partners in various branches of the Centers for Disease Control and Prevention (CDC) for their support and collaboration, including the Division of Vector-Borne Infectious Diseases (DVBID) in the National Center for Zoonotic, Vector-Borne, and Enteric Diseases (ZVED), and the Influenza and Viral Diseases Divisions in the National Center for Immunization and Respiratory Diseases (NCIRD);

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Dr. Larry Dickey at the CDPH Committee for Protection of Human Subjects for his advice; and

The many physicians who refer cases to the VRDL for testing.

Letter from the Branch Chief

Dear Public Health Partners,

It has been nearly three decades since the Viral and Rickettsial Disease Laboratory (VRDL) issued an annual report. Since that time, the VRDL has undergone extensive changes in its leadership, infrastructure, priorities and staffing. The intention of this report is to provide an overview of VRDL's major services and activities. This report will focus on diagnostic testing advances, surveillance projects, and research developments in 2007 and 2008.

Until recently, the VRDL was part of the California Department of Health Services (CDHS). In July 2007, CDHS was reorganized into two new departments: the California Department of Public Health (CDPH) and the Department of Health Care Services (DHCS). The main purpose of the reorganization was to provide more focused leadership in public health and health care financing and create a more effective public health infrastructure in California. As part of the new CDPH, VRDL is dedicated to providing the scientific leadership essential to this public health infrastructure.

One of the focuses of VRDL in the last few years has been to provide leadership to develop surge capacity among public health laboratories throughout the state for potential public health emergencies such as pandemic influenza. In 2003, when the global epidemic of severe acute respiratory syndrome (SARS) came without warning, the limited capacity for respiratory virus detection within the state became apparent. Since that time, the VRDL has worked closely with local public health laboratories to develop the Respiratory Laboratory Network, as well as the West Nile Virus Regional Public Health Laboratory Network. Twenty-five local public health laboratories participate in the Respiratory Laboratory Network by offering enhanced diagnostic testing for respiratory viruses, while over 30 local laboratories are able to perform West Nile virus testing as part of the West Nile virus regional public health laboratory network.

VRDL has had several areas of achievement, including the development of surveillance programs for influenza, norovirus, and West Nile virus; development of new testing for influenza antiviral resistance and Chikungunya virus; the California Respiratory Project; the Unexplained Death Project, and the inception of the California Encephalitis Project.

Sincerely Yours,

Carol Glaser, D.V.M., M.D.
Chief, Viral and Rickettsial Disease Laboratory

Introduction

The mission of the California Department of Public Health (CDPH) Viral and Rickettsial Disease Laboratory (VRDL) is to provide laboratory and scientific expertise essential for the detection, epidemiologic investigation, and control of viral and rickettsial agents associated with communicable diseases and disease-related outbreaks in California. The VRDL provides primary and reference laboratory support to all 37 local public health laboratories (LPHLs) in California and some reference services to clinical laboratories in the state. Laboratory support is also provided to counties without public health laboratory services.

Under the guidance of the Branch Chief Dr. Carol Glaser and the Assistant Branch Chief Kimberly Ferreira, the VRDL operates with a staff of approximately 100 individuals. VRDL has been located at a new laboratory complex housed at the CDPH campus in Richmond, California since 2001. The VRDL Branch is divided into five sections: (1) the Viral Immunoserology & Molecular Diagnostic Section, (2) Viral Isolation, (3) the Retrovirus Diagnostic Section, (4) Epidemiology Support, and (5) the Medical and Epidemiology Liaison Section.

While the Viral Immunoserology & Molecular Diagnostic, Viral Isolation, Retrovirus Diagnostic and Epidemiology Support sections work to provide specialized expertise and services for their respective areas, they also collaborate on specific activities, sharing staff and skills to serve the overall mission of the branch. Some examples of these cross-section collaborations include diagnostic and surveillance activities, public health microbiology training, laboratory information systems development and molecular testing development. The Medical and Epidemiology Liaison Section works to support the diagnostic services of the other sections by coordinating diagnostic testing [e.g., medical screening, record-keeping, biosafety, and adherence to Clinical Laboratory Improvement Amendments (CLIA) and Health Insurance Portability and Accountability Act (HIPAA) standards], as well as organizing and managing specific surveillance and training activities.

The services provided by VRDL can be divided into several broad areas:

I. **Diagnostic testing services**
   a. Performs viral and rickettsial disease testing for surveillance and investigation
   b. Performs test services for contract local health departments and other agencies without public health laboratory services of their own (e.g. primary rabies testing, viral isolation)
   c. Provides reference and specialized tests beyond local capabilities
   d. Provides technical consultation to laboratorians and infectious disease clinicians on complex viral diseases, especially in the area of diagnosis
   e. Offers multiple methods of diagnostic testing, as well as testing for reseach, surveillance and epidemiologic purposes, including:
      - Isolation of multiple viruses
      - Serologic testing
        - Indirect fluorescent antibody (IFA) and direct fluorescent antibody (DFA) for multiple viruses including West Nile virus (WNV), rabies, respiratory viruses, mumps, varicella, herpes viruses, pox viruses,

human immunodeficiency virus (HIV), human T-cell leukemia virus (HTLV), and rickettsiae
  - Enzyme immunoassay (EIA) for multiple viruses including WNV and other arboviruses, measles, mumps, rubella, varicella, hepatitis, HTLV, HIV, and Sin Nombre virus (SNV-hantavirus)
  - Western blot for HIV, HTLV, and WNV
  - Serotyping for enteroviruses, adenoviruses, and rhinoviruses
  - Influenza straintyping
- Molecular testing [polymerase chain reaction (PCR), sequencing, etc.] of respiratory viruses, herpesviruses, rabies, pox viruses, norovirus, enterovirus, HTLV, and HIV
- Antiviral resistance testing for HIV, influenza, and other agents

II. Specialized testing of outbreaks and unusual diseases

a. Bioterrorism viral agents (e.g., smallpox)
   - Works closely with the LPHLs to incorporate varicella and vaccinia testing within the LPHLs
   - Acts as a beta-test site for variola testing when needed
b. Dengue and Chikungunya
   - Works closely with the CDC in providing confirmatory testing for these emerging viruses in travelers, as well as developing sensitive and specific in-house testing.
c. Hantavirus pulmonary disease syndrome
   - Serves as the only public health laboratory in the state providing testing for SNV
d. Outbreaks of respiratory illness
   - Offers molecular testing for a large panel of respiratory viruses to determine etiology and assist with containment of institutional outbreaks
e. Tick-borne disease
   - Provides IFA testing for several rickettsial diseases [Rocky Mountain Spotted Fever (RMSF), typhus, Q fever, and ehrlichiosis].
f. Vaccine-preventable diseases
   - Provides definitive testing with serology and PCR for rash illnesses such as measles, varicella and rubella (e.g. statewide reference testing for mumps during a large mid-west US outbreak)

III. Surveillance of viral diseases of public health importance

a. California Encephalitis Project (CEP)
   - Plays a key role in the CEP, which is a program designed to provide state-of–the-art diagnostic testing for cases of human encephalitis
   - Through enhanced diagnostic testing, the CEP enables better understanding of encephalitis in humans, including risk exposures, symptomatology, and clinical features
   - Validation of an effective testing algorithm for encephalitis is a critical component
b. California Influenza Surveillance Project (CISP)
   - Monitors statewide influenza activity each season, and provides essential clinical and laboratory information about trends in influenza-like illness and circulating influenza strains, especially with reference to new or emerging epidemic or pandemic strains

c. Gastrointestinal disease outbreaks
   - Offers molecular tests on specimens from acute viral gastroenteritis outbreaks and can rapidly identify Norwalk-like agents
   - Performs molecular characterization of a subset of isolates to monitor trends in circulating strains throughout the state.

d. Rabies
   - Serves as the reference laboratory for all LPHLs for rabies testing of suspect animal cases (VRDL is the only laboratory in California able to perform testing for human rabies cases)

e. Unexplained Deaths Surveillance (UNEX)
   - Works with MDL to provide assistance for diagnostic workup of fatalities due to possible infectious etiology
   - A broad range of diagnostic testing for viral, bacterial and rickettsial pathogens is available, including molecular-based assays such as PCR and sequencing, culture and serologic testing

f. West Nile virus
   - Performs testing in support of human surveillance
   - Has played a key role in incorporating WNV screening tests at local laboratories throughout the state
   - Has played a key role in consulting with local health departments on suspect human WNV cases

IV. Support of local public health laboratories:

a. Provides reference test services to assist LPHLs with confirmatory testing with diagnostic tests, disease surveillance, and epidemiologic studies which are too hazardous, difficult or unusual for them to perform
b. Evaluates and standardizes methods and reagents for local use
c. Evaluates local test performance

V. Virology training for local laboratory personnel and doctoral fellows, including:

a. Participation in the Public Health Microbiology Training Program
b. Year-round provision of training for LPHL laboratorians to incorporate testing of emerging agents of key public health concern (such as influenza, avian influenza, WNV, norovirus, etc.)

VI. Research and development activities, including:

a. Development, evaluation and standardization of novel diagnostic procedures (e.g. oral HIV test, neutralization tests for WNV, HIV, and avian influenza, Western blot assays for Chikungunya, dengue, and other flaviviruses, protein microarrays, low cost HIV assay development for use in resource-poor settings, novel culture systems, multiplex detection assays, and new diagnostic assays for emerging infections such as human herpes virus-6 (HHV-6), avian influenza A (H5N1), mumps, coronaviruses and bocaviruses)

VII. Other activities in support of state and local partners, including:

a. Collaboration with the CDPH Vector-Borne Disease Section (VBDS) in testing rodents for SNV

b. Collaboration with the CDPH Veterinary Public Health Section in testing wild animals for rabies
c. Works closely with the Office of Acquired Immunodeficiency Syndrome (AIDS) in performing HIV surveillance studies.
d. Ongoing collaborative efforts with many other academic, commercial and public health partners, including Cepheid Corporation, Diagnostic Hybrids, Inc, University of California Davis, University of California Irvine, University of California Los Angeles, University of Iowa, University of Texas and the World Health Organization (WHO).

Section Activities

- **Viral Immunoserology and Molecular Diagnostic Section**
- **Viral Isolation Section**
- **Retrovirus Diagnostic Section**
- **Epidemiology Support Section**
- **Medical & Epidemiology Liaison Section**

Viral Immunoserology and Molecular Diagnostic Section

Section Chief: Dr. Sharon Messenger

Background

The Viral Immunoserology and Molecular Diagnostic Section participates in a broad range of diagnostic testing that includes EIA testing and molecular testing for multiple infectious diseases in humans, including arboviral, respiratory and rash illnesses. The Viral Immunoserology and Molecular Diagnostic Section plays a key role in providing state-of-the-art diagnostic testing for the California Encephalitis Project as well as the primary testing performed for the WNV Surveillance Project. In addition, tests performed in this section are critical for detection of vaccine-preventable diseases such as measles, mumps, rubella and varicella.

Training

Viral Immunoserology and Molecular Diagnostics staff members play a major role in didactic teaching and on-hands training at the bench for the VRDL Public Health Microbiologist Training class.

Laboratory Testing, Diagnostic Services and Reference Testing

- **In-house EIA testing**
  - EIA testing is a sensitive analytical assay used:
    - To identify acute cases of viral disease
    - To demonstrate the presence of antibody as an indication of chronic infection
    - To measure immunity status relating to vaccine-preventable diseases
    - For epidemiology- prevalence and incidence studies

  - In-house EIA testing is used to identify causative agents of infectious disease:

    Respiratory infections: Influenza A, Influenza B, *Mycoplasma pneumoniae*, Adenovirus, Respiratory Syncytial virus, and parainfluenza

    Encephalitis and Aseptic Meningitis: Measles, herpes simplex virus (HSV), varicella zoster virus (VZV), St. Louis Encephalitis, WNV, and *M. pneumoniae*

    Rashes, Morbilliform or Maculopapular: Rubella and measles

    Rashes, Vesicular: HSV and VZV

    Congenital: Cytomegalovirus, rubella, and HSV

    Genital: HSV and *Chlamydia* group

The Viral Immunoserology and Molecular Diagnostic Section has the ability to test for IgM against 14 analytes and for IgG against 17 analytes known to be associated with the above clinical syndromes.

- **Other EIA assays** performed include WNV IgM, Dengue IgM, and *Mycoplasma* IgM commercially-available assays

Table 1. Enzyme immunoassay testing using in-house EIA assay, 2007.

Analyte	# Specimens tested	
	IgM	IgG
Rubella	21	42
Measles	40	463
Herpes simplex virus	213	302
Varicella zoster virus	112	257
Cytomegalovirus	6	9
Mumps	31[a]	25
St. Louis encephalitis	17	25
Western equine encephalitis	17[a]	198
Dengue	b	99
Adenovirus	20	162
Mycoplasma pneumoniae	c	361
***Chlamydia* sp.**	19	81
Influenza A, B	-	136
West Nile virus	d	954

a. Discontinued test.
b. IgM antibodies to Dengue Virus are tested using a commercial kit. 161 samples were tested in 2007.
c. IgM antibodies to *Mycoplasma pneumoniae* are tested using a commercial kit. 683 samples were tested in 2007.
d. IgM antibodies to West Nile Virus are tested using a commercial kit. 1586 samples were tested in 2007.

- **Molecular diagnostic testing**
  - Molecular diagnostic testing performed by the section includes real-time reverse transcriptase (RT)-PCR for detection of viruses that are of public health concern

    Herpesviruses: Real-time TaqMan-based PCR testing for a panel of herpesviruses, including HSV-1, HSV-2, VZV, Epstein-Barr virus (EBV), and HHV-6. This panel is performed in large part to support the California Encephalitis Project. In 2007, cerebrospinal fluid for 597 patients was tested against this panel of herpesviruses.

    Respiratory viruses: Real-time PCR is used as a rapid surveillance and diagnostic tool for the detection of respiratory diseases, including influenza A and B, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and adenoviruses for surveillance of pandemic and avian influenza. The VRDL works closely with local and county public health labs and medical professionals to assist in the diagnostic testing and surveillance of patient specimens from institutional or community respiratory outbreaks, individual

cases of severe respiratory illness, cases of suspected infection with avian (H5N1) influenza, as well as cases of concern from physicians, private hospital and LPHLs.

Table 2. Respiratory panel real-time RT-PCR testing in 2007.

Analyte	# Specimens positive (N tested=462)
	Nucleic acid detected
Respiratory syncytial virus	29
Influenza A	44
Influenza B	6
Human metapneumovirus	12
Adenovirus	17
Total	108

Special Diagnostic and Research Projects:

- Avidity testing
  - West Nile virus IgM antibody testing has been the diagnostic test of choice for determining recent infection in humans, but IgM has been found to persist in some human cases for over a year
  - An IgG avidity test is used to aid in the diagnosis of WNV infection by distinguishing between recently acquired infections and past infections
  - As IgG antibodies mature over time their net binding forces increase, we use the measurement of the binding force percentage to aid in diagnosis and surveillance of acute WNV infections

- Confirmatory testing for Chikungunya virus in travelers, in collaboration with the CDC

- Development of sensitive and specific in-house testing for both the Chikungunya virus as well as dengue virus, which has a similar clinical presentation to Chikungunya, and is endemic in the same areas

- Testing for a large panel of respiratory viruses during outbreak situations

- Testing for rash illnesses such as measles, varicella, and rubella during outbreak situations

- Development and validation of rapid molecular real-time PCR assays for febrile rash illnesses (i.e., measles and mumps) and respiratory viruses (i.e., parainfluenza viruses 1-4, which will be added to the respiratory real-time PCR panel in 2008)

- Confirmatory EIA and neutralization tests for WNV as a service to state public heath departments in Nevada and Oregon (funded by a grant from CDC)

External Funding and Grant Support

MMR2 Study: The Immunoserology and Molecular Diagnostics Section is in the 13th year of a continuing collaborative study with CDC Immunization Branch to evaluate the persistence of antibody to mumps, measles and rubella following MMR immunization of school age children. In 2007, the Section tested 1282 samples for immunity to rubella by EIA and neutralization. An additional 150 samples were received at the end of the year that will be tested in 2008 for immunity to mumps and rubella by EIA and neutralization. These samples are the final samples from the study which is scheduled for completion and publication in 2008.

GlaxoSmithKlein Project: In this project the Immunoserology and Molecular Diagnostics Section is performing Varicella Zoster Virus Fluorescent Antibody Membrane Assay (FAMA) testing to determine the effect of other vaccines on the antibody response to VZV Vaccine. The VZV FAMA test is a specialized test for antibody that is performed in very few labs worldwide. We have developed an efficient method of performing this test that is unique to the VRDL. In 2007, the Section tested 1752 samples by VZV FAMA for various vaccine clinical trials. This testing will continue through 2008.

Viral Isolation Section

Section Chief: Dr. David Schnurr

Background

The Viral Isolation Section participates in a broad range of diagnostic testing for infectious diseases in humans that includes viral isolation and identification, antibody detection by indirect immunofluorescence antibody (IFA) testing, DFA testing, and PCR. Special areas of interest include surveillance and research for influenza and other respiratory viruses, rabies, norovirus, enterovirus and rickettsiae.

The Viral Isolation Section plays a key role in laboratory-based surveillance for influenza and other respiratory viruses in California. In 2007, the Section tested over 1,000 specimens for respiratory agents, including over 500 from the CDC Influenza Sentinel Provider Influenza Surveillance Program. The Section actively participates in numerous collaborations. In conjunction with other VRDL sections, Viral Isolation staff members have worked to adapt molecular assays for newly discovered respiratory viruses, including human metapneumovirus and bocavirus. They have also worked with other sections to adapt assays for influenza typing and subtyping, including assays for avian influenza A (H5N1). Finally, Viral Isolation staff members have also been instrumental in distributing reagents and training partner LPHLs in performing influenza PCR and utilizing enhanced culture systems to test for multiple respiratory viruses at the local level. The creation of the Respiratory Laboratory Network, a network of 22 LPHLs performing this enhanced diagnostic testing, has resulted in a significant increased capacity of the public health laboratory system in the state as a whole to test for a large panel of viral agents in preparation for an influenza pandemic or other epidemic due to emerging respiratory viruses (e.g. Severe Acute Respiratory Syndrome, or SARS). (More information on 2007 influenza surveillance can be found in the Section on Laboratory-Based Surveillance Projects under the California Influenza Surveillance Project as well as at: http://www.cdph.ca.gov/programs/vrdl/Pages/CaliforniaInfluenzaSurveillanceProject.aspx.)

The Viral Isolation Section is also responsible for testing of acute viral gastroenteritis outbreaks (e.g. norovirus) and for distributing reagents and training partner LPHLs in performing norovirus PCR. In addition, further analysis of selected specimens is performed in order to track the molecular epidemiology of norovirus throughout the state, and its association with different populations, settings and types of outbreaks. (More information on acute viral gastroenteritis surveillance can be found in the Section on Laboratory-Based Surveillance Projects under Viral Gastroenteritis (Norovirus) Outbreaks).

One of the most important services offered by the Viral Isolation Section is rabies testing. The VRDL serves as the reference laboratory for the state of California, as well as the primary rabies laboratory for several California counties that do not have a public health laboratory.

For suspect cases of rabies in humans, the VRDL offers ante and post-mortem testing. Ante-mortem assays include DFA (nuchal biopsy or corneal impressions), PCR (saliva, nuchal biopsy or corneal impression), and culture of saliva. Serologic testing is also performed using either IFA or with neutralization assay using the Rabies Fluorescent

Focus Inhibition Test (RFFIT). In 2007, a single case of laboratory-confirmed human rabies occurred in California. There was history of possible exposure to rabies due to a dog or fox bite in Mexico. The case was diagnosed as rabies by DFA at the LPHL and brain specimen was sent to the VRDL, where the strain was determined to be from a bat.

Although human rabies testing is offered, the VRDL primarily tests brain tissue from domestic animals, or animals that have been in contact with humans, to determine the risk of rabies exposure and transmission. The primary diagnostic test for rabies in animals is the DFA assay. In 2007, 4.8% (9/186) animal brains tested were positive for rabies by DFA, including 4 bats, 3 skunks, a cat and a raccoon. For reasons that are unclear, the number of specimens submitted for testing and the number of rabies positive specimens has decreased in recent years; it is possible that the incidence of rabies overall in California, particularly in skunks, may be decreasing. Other diagnostic tests offered for rabies at the VRDL are viral culture, antibody assay by IFA and neutralization and as a complementary assay, PCR. In addition, animals are sometimes tested for surveillance purposes only, i.e. when no human exposure has occurred (More information on animal rabies surveillance can be found in the Section on Laboratory-Based Surveillance Projects under California Rabies Surveillance Program).

Training

Viral Isolation staff members play a major role in didactic teaching and on-hands training at the bench for the VRDL Microbiologist Training class. In 2006 the VRDL, along with the National Laboratory Training Network and the CDC, hosted a two day workshop entitled “Rabies training: Laboratory Methods for Detecting Rabies Virus”. The course is scheduled to be offered again in March 2009. On a regular basis public health microbiologists from LPHLs visit the VRDL for training. VRDL staff also travel to LPHLs to provide specialized training, especially in influenza and norovirus PCR. Staff from other laboratories around the world frequently consult with or visit the Section for discussion, training and collaboration on numerous projects.

Laboratory Testing, Diagnostic Services and Reference Testing

- **Viral culture**
  - Viral culture is performed on a variety of human specimens using in-house and commercial cell cultures
  - Although particular viruses may be targeted, one important advantage of culture is that any virus that is present in the specimen that can grow in the culture system may be amplified and identified; thus, a wide range of viruses may be detected

  Respiratory specimens
  - Influenza. From January - December 2007, approximately 1300 respiratory specimens from various settings, including the CDC Influenza Sentinel Provider Surveillance Program, the California Encephalitis Project, surveillance of intensive care units, institutional outbreaks, and individual cases of severe respiratory illness were processed (Tables 1-3). A virus was isolated from 438 specimens, for an overall isolation rate of 31.5 %. The majority (>75%) of the isolates were influenza. Specimens from the CDC Influenza Sentinel Provider Surveillance Program were especially productive, with over half positive for a respiratory virus.

– Besides influenza, other isolates of public health importance were also identified, including enterovirus 71, mumps, and adenovirus 14. Adenovirus 14 is recognized as a newly emerging adenovirus variant in the United States, associated with severe disease and outbreaks in military and hospital settings. Through ongoing surveillance efforts, the VRDL was the first to identify cases of severe respiratory illness due to adenovirus 14 in the US in 2004 and 2005.

Table 1. Respiratory Isolation Specimens from Various Projects, 2007

Project	Total	Positive	Negative	Unsat	% Positive
Sentinel Physician Project	554	335	219	0	61%
Encephalitis Project	381	23	358		6.0%
Outbreaks, other projects	354	80	274	0	22.5%
Total	1289	438	851		34%

Table 2. Respiratory Isolation Specimens by Agent, 2007

Agent	# Identified	Comments
Respiratory syncytial virus	5	
Adenovirus 1	1	
Adenovirus 14	3	
Adenovirus 2	3	
Adenovirus 3	9	1 dual infection with parainfluenza virus 3
Adenovirus 4	3	
Coxsackievirus A9	1	
Coxsackievirus B1	1	
Echovirus type 11	1	
Echovirus type 3	1	
Echovirus type 9	1	
Enterovirus 68	3	
Enterovirus 71	1	
Human metapneumovirus	2	
Herpes simplex virus 1	11	
Influenza A (H1)	163	1 dual infection H1/H3
Influenza A (H3)	89	
Influenza A (no subtype)	75	
Influenza B	20	
Mumps	2	
Parainfluenza virus type 1	17	
Parainfluenza virus type 2	1	
Parainfluenza virus type 3	14	
Parainfluenza virus type 4	1	
Paraechovirus	1	
Rhinovirus	24	1 dual infection HSV-1/Rhinovirus

Non-respiratory specimens

– Specimens in this category include sterile body fluids (e.g. cerebrospinal fluid, urine, skin biopsies and tissue samples) as well as viral isolates forwarded from another laboratory for further culture identification. Twenty-two enteroviruses were typed including 8 Coxsackie B1 viruses, the most frequently identified enterovirus in 2007. Other isolates included a number of herpes viruses, adenoviruses and mumps.

Table 3. Non-respiratory Specimen Identification by Agent, 2007

Agent	# Identified	Comment
Adenovirus type 1	1	
Adenovirus type 2	2	
Adenovirus type 8	2	
Cytomegalovirus	1	Source: urine
Coxsackievirus B1	8	
Coxsackievirus B4	2	
Coxsackievirus B5	1	
Echovirus type 25	1	
Echovirus type 4	1	
Echovirus type 14	2	
Echovirus type 15	1	
Echovirus type 18	3	
Echovirus type 2	1	
Echovirus type 5	1	
Echovirus type 9	1	
Herpes simplex virus 1	4	
Herpes simplex virus 2	4	
Mumps	2	
Parainfluenza virus type 1	1	Source: urine
Rhinovirus	3	Source: autopsy tissue

- **Immunofluorescent Assay (IFA) for Human IgG and IgM**
  - The IFA is a serologic assay that can be run for any agent that can be cultured using cells grown *in vitro.* The assay is relatively labor-intensive and requires skilled subjective interpretation. At the VRDL the IFA is used as the primary diagnostic test for detection of antibodies to mumps, rickettsiae and unusual agents such as Orf virus. IFA is also utilized as a confirmatory assay to ELISA for WNV, dengue and other arboviruses. In 2007 the Viral Isolation Section ran more than 1,000 IFA assays (see Table 4). Some highlights were the detection of *Rickettsiae typhii* (causes tick typhus), *Anaplasma phagocytophilum* (causes human granulocytic erlichiosis or HGE), dengue, mumps and measles. A sensitive, specific IFA assay for antibodies to Chikungunya virus was also developed and validation is in progress.

Table 4. IFA Assays for Human IgG and IgM by Agent, 2007

Agent	# Tested	Significant Findings*
Chlamydia psittaci	1	
Chlamydia trachomatis	1	
Rickettsial panel	118	
- *Rickettsia rickettsii* (RMSF)	34	3 G/M+(1-also had high titers to *R. africae*)
- *R. typhi*	11	6 G/M+
- *Ehrlichia chaffeensis* (HME)	3	1 G/M+
- *Anaplasma phagocytophilum* (HGE)	18	2 G/M+
- *Coxiellla burnetti* (Q fever)	25	
Dengue	70	1 seroconversion; 4 G/M+
St. Louis encephalitis	3	
West Nile virus	160	60 G/M+
Mumps	61	24 specimens CDC PT study; 4 G/M+
Rabies	22	
Lymphocytic choriomeningitis virus	17	
Measles	10	1 G/M+
Parvovirus	498	2 @ >=80 titer;3 @ >=160 titer
Western equine encephalitis	1	
Chikungunya	33	3 G/M+

* G/M+ = both IgG and IgM identified in serum

- **Polymerase Chain Reaction (PCR) Testing (of Humans)**
  - The Viral Isolation Section provides routine diagnostic PCR for norovirus, enterovirus, rhinovirus, mycoplasma, influenza, and other respiratory viruses, as well as a complementary assay for rabies diagnosis. PCR is also used as an epidemiologic tool for various surveillance projects:

    Norovirus: Real-time PCR for the diagnosis of norovirus in outbreak setttings is offered. In 2007-08, 38 of 47 (81%) of outbreaks where testing of stool specimens was performed were norovirus-positive and 148 of 239 (62%) of individual stool specimens were positive. More information on norovirus can be found in the Section on Laboratory-Based Surveillance Projects under Viral Gastrointesteritis (Norovirus) Outbreaks.

    Enterovirus: PCR has become the standard assay for diagnosing enterovirus infections from cases of encephalitis and meningitis. In 2007 the VRDL identified enterovirus in 13 of 630 (2%) persons with suspected encephalitis whose specimens were submitted to the California Encephalitis Project, and as an alternative diagnosis in 6.6% (39/588) of cases submitted for WNV testing (see Table 5).

Rhinovirus: PCR is considerably more sensitive than culture, the gold standard for detection of rhinovirus. In 2007 129 of 413 (31%) of respiratory specimens tested were rhinovirus positive. PCR is also used to confirm presumptive culture-positive rhinoviruses.

Table 5. PCR Testing for Enterovirus, 2007

Specimen Type		Total #	Pos	Neg	% Pos
All		1811	75	1736	4.14
CSF	All	1218	52	1166	4.27
	CEP*	630	13	617	2.06
	WNV	588	39	549	6.63
Respiratory		544	17	527	3.13
Tissue		25	0	25	0.00
Other		24	6	18	25.00

* California Encephalitis Project

Influenza and other respiratory viruses: The Viral Isolation Section, in conjunction with staff from other VRDL sections, plays a major role in the adaptation, implementation and validation of molecular testing for influenza and other respiratory agents, including influenza A and B, RSV, adenovirus, human metapneumovirus, parainfluenza viruses 1-3 and picornavirus, as well as *M. pneumoniae.* In collaboration with local health departments, rapid molecular testing is utilized to test individual cases of severe respiratory illness, pediatric cases hospitalized in intensive care, and from institutional outbreak settings.

Novel influenza virus testing: The VRDL assists local health departments by offering testing for persons who meet the definition for suspect avian influenza A (H5N1) (information available at http://www.cdph.ca.gov/programs/vrdl/Pages/EnhancedSurveillanceforAvianInfluenzaA(H5N1).aspx). Testing of suspect cases who meet criteria can be performed by real time PCR, with turn-around time of approximately 6 -8 hours once specimens arrive, and with pre-arrangement with local health departments. Other complex testing that is also available includes antiviral drug resistance testing, strain characterization, and sequencing. The Viral Immunoserology & Molecular Diagnostic Section is developing micro-neutralization assays for testing of antibody levels to influenza A (H5N1).

<u>Special Diagnostic and Research Projects</u>:

- Validation of real-time PCR for influenza A and B, H1, H3 and H5 (collaboration with CDC)
  - VRDL is one of seven state public health laboratories which participated in this CDC validation study

  - Following validation these assays will be available to all Laboratory Response Network laboratories for testing specimens for pandemic influenza

- Genotyping of human rhinoviruses
  - Molecular typing of rhinoviruses is problematic because there are over 100 known serotypes and because rhinoviruses are difficult to grow in culture (the amplification of sequences from the capsid proteins requires cultured virus)
  - The Viral Isolation section has developed a method for serotyping human rhinoviruses based on sequences in the 5'noncoding region that can be applied to original specimens

- Development of new culture systems for influenza
  - The Viral Isolation Section is working on methods for the sensitive detection and efficient growth of influenza viruses by using MDCK cells which are genetically engineered to have increased expression of the receptor for human influenza virus
  - When compared to unmodified MDCK cells, these genetically engineered MDCK cells are superior with regard to sensitivity, rapid detection and subtyping of influenza viruses; they were found to be very sensitive when used for culture of sentinel physician specimens in the 2007 -08 influenza season

- Characterization of intermediate adenoviruses (collaboration with the University of Iowa, National Adenovirus Surveillance System)
  - A system for molecular typing of hexon and fiber proteins has been developed and validated

External Funding and Grant Support

Epidemiology and Laboratory Capacity for Infectious Diseases: National Center for Infectious Diseases (NCID) Grant Number: 5 U50 C1923677003 (PI: D. Schnurr).

Grant from the Association of Public Health Laboratories (APHL) Inc. for Reagent Evaluation: Comparison of the Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel with Virus Culture for Human Respiratory Specimens from Patients Presenting with Influenza-like Illness.

Retrovirus Diagnostic Section

Section Chief: Dr. Carl Hanson

Background

As a statewide reference laboratory, the Retrovirus Diagnostic Section is responsible for performing an algorithm of confirmatory serologic assays for human immunodeficiency virus (HIV) in humans. Serologic testing for human HTLV infection is also provided for county public health laboratories, hospital laboratories, blood centers and other reference laboratories. Development of new assays, special short-term diagnostic projects and grant-supported applied research is also conducted. With the advent of bioterrorism and the naturally occurring epidemic of WNV, it was recently recognized that techniques used in retroviral diagnostics could be advantageously applied to other viral diseases, thus leading to an expansion of this Section's activities into serologic diagnosis of infections by WNV, and other domestic and exotic emerging arboviruses.

Training

Retrovirus Diagnostic Section staff teach in the VRDL Microbiologist Training class. In addition, the Section has been fortunate to have been assigned each of the past five years an Emerging Infectious Diseases fellow, funded by the CDC and the Association of Public Health Laboratories (APHL) and selected in a nationwide competition. Staff members from other laboratories around the world also frequently visit the Section for discussion, training and collaboration.

Laboratory Testing, Diagnostic Services and Reference Testing

- **HIV-1 screening and confirmatory testing (EIA, IFA, and Western blot)**
  - Routine diagnostic testing on samples from county and hospital laboratories
  - Samples are generally diagnostically difficult samples referred to CDPH/VRDL for further testing and are from counties without a public health laboratory
  - Number of samples (2007): 358 tested; 32 confirmed positive
  - Test algorithm:
    All specimens sent for HIV-1 testing are screened using a licensed EIA kit and an in-house IFA simultaneously. If the results from these two tests are concordant, an overall interpretation of antibody detected or not detected is reported. When the EIA and IFA results are discrepant or the IFA is unsatisfactory (nonspecific), an in-house Western blot is performed. Two assay methods must be concordant before VRDL reports antibody detected or antibody not detected. When no two test results are concordant the overall interpretation is "inconclusive".

- **HIV-2 screening and confirmatory testing** (EIA, IFA and Western blot)
  - Routine diagnostic testing on samples from county laboratories
  - Samples from patients having risk factors for HIV-2 or samples which are HIV-1/-2 EIA reactive samples that require further testing to rule out HIV-2
  - Number of samples (2007): 196 tested; 39 confirmed positive
  - Persons at risk for HIV-2 infection include:
    - Persons from a country where HIV-2 is endemic (e.g. in Africa)
    - Sex partner(s) of a person from a country where HIV-2 is endemic

  - Sex partner(s) of a person known to be infected with HIV-2
  - Received a blood transfusion or a nonsterile injection in a country where HIV-2 is endemic
  - People who shared needles with a person from a country where HIV-2 is endemic or with a person known to be infected with HIV-2
  - Children of women who have risk factors for HIV-2 infection or are known to be infected with HIV-2

  Testing is also indicated for:
  - People with an illness that suggests HIV infection (such as an HIV-associated opportunistic infection) but whose HIV-1 test result is not reactive
  - People for whom a HIV-1 Western blot exhibits a specific unusual indeterminate test band pattern

- Test algorithm:
  If travel history or exposure history support HIV-2 testing, the specimen is routed for HIV-2 testing by EIA and IFA. If EIA and IFA results are concordant, they are reported to the submitter. If results are discordant, the sample is tested by Western blot. Local lab HIV-1/HIV-2 combi EIA reactive samples confirmed locally as HIV-1 negative or indeterminate are tested by HIV-2 EIA. If the HIV-2 EIA is reactive the result is confirmed by HIV-2 IFA. Local lab "combi" EIA reactive specimens not confirmed locally for HIV-1 are tested by HIV-1 EIA and IFA. If results are discordant, the sample is tested by Western blot. If EIA and IFA assays do not detect HIV-1 antibody, the specimen is routed for HIV-2 testing.

- **HTLV-I/HTLV-II screening and confirmatory testing** [EIA, IFA, Western blot and Radioimmunoprecipitation Assay (RIPA)]
  - HTLV-I is a cause of adult T-cell leukemia/lymphoma and tropical spastic paraparesis. HTLV-II, which currently has no confirmed health effects, is highly prevalent in injection drug users (up to 49% in some groups), and serologically difficult to distinguish from HTLV-I.
  - Of 2625 HIV-infected American women enrolled in the Women's Interagency HIV Study (see below), 10% were infected with HTLV-II and 0.5 % with HTLV-I. While HTLV-I infection can enhance HIV infection, HTLV-II infection actually slows HIV disease progression.
  - In 1988, the Food and Drug Administration (FDA) recommended that blood donated for transfusion in the United States be screened for antibodies to HTLV (now implemented by all blood centers in the US). Persons seropositive for HTLV-I/II should neither donate blood for transfusion nor share needles. Women should be advised against breast feeding, and individualized advice about sexual activity should be given. To minimize anxiety in counseling persons found to be seropositive for HTLV-I/II, the American Red Cross and a number of other blood centers and reference laboratories contract with the VRDL to distinguish serologically between HTLV-I and HTLV-II, because of the very different (and untreatable) disease associations of these two virus types.
  - Test algorithm:
    All specimens sent to VRDL for HTLV-I/II testing are screened by an FDA approved EIA kit and an in-house IFA simultaneously. If the results from

these two tests are concordant, an overall interpretation of antibody detected or not detected is reported. The IFA must react with both HTLV-I and HTLV-II antigens to be considered reactive. A reaction on one antigen and not the other is considered inconclusive. Reactive specimens are typed by IFA endpoint titration to determine if the specimen is HTLV-I or HTLV-II. When the EIA and IFA results are discrepant or the IFA is unsatisfactory (nonspecific) or inconclusive, an in-house Western Blot is performed. If two test results are concordant, we report that result as an overall interpretation (antibody detected or not detected). If no two test results are concordant an in-house RIPA is performed. After RIPA, interpretation is as follows: If two test results are now concordant, the result is reported as antibody detected or not detected. If no two test results are concordant, the overall interpretation is inconclusive. Due to the weakness and pattern of the antibody response, some samples having an EIA reactive, IFA nonreactive and Western Blot positive result may be further tested by RIPA. The overall interpretation is antibody detected (not typed) when the RIPA is positive and inconclusive when the RIPA is negative.

- Confirmation and typing for hospitals, diagnostic labs, and blood centers: Most samples sent to VRDL for HTLV-I/II testing have been previously screened and found reactive by one or two EIAs. As there are no FDA-approved confirmatory assays, samples are sent to VRDL for confirmatory testing. Approximately 74% of samples are not confirmed as positive. Our results are used to counsel blood donors, assist physicians involved with transplants and at infertility clinics, and new mothers who screen reactive and want to breastfeed. The reason for testing is to stop transmission.
- Number of samples (2007): 2,937 tested; 582 confirmed positive

- **Epstein-Barr virus serology** (IFA for IgG, IgM, and EBNA antibody)
  - 431 samples tested, primarily for the California Encephalitis Project

- **Arbovirus Serology**
  - NIH Pacific Southwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases (PSWRCE): Dr. Hanson is a Project Director of the PSWRCE. In 2005, NIAID completed a national network of ten Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCEs), to support research focused on countering threats from potential bioterrorism-related agents and emerging infectious diseases. Each Center is comprised of a consortium of universities and complementary research institutions serving a specific geographical region. The primary objective of the RCE program is to support the NIAID Biodefense and Emerging Infectious Diseases Research Agenda. The RCEs, located throughout the United States, build and maintain a strong scientific infrastructure supporting multifaceted research and development activities that promote scientific discovery and translational research capacity required to create the next generation of therapeutics, vaccines, and diagnostics for the NIAID Category A-C Priority Pathogens (PDF)

- **WNV Serology**
  - Partly as a portion of its work with the PSWRCE, the Retrovirus Diagnostic Section has developed a Western blot assay for confirmatory testing of

antibodies to WNV and the related St. Louis encephalitis virus. This assay reduces the time required for confirmatory testing from 10 days to 1 day.

- **Other Arboviruses**
  - The Retroviral Diagnostic Section is continuing to develop Western blot and related serologic assays for use with Chikungunya virus, dengue virus and other arboviruses. Recently validated for human diagnostic use, the new Western blot for Chikungunya virus correctly identified this infection in travelers returning to California from India.

Special Diagnostic and Research Projects:

- **HIV viral load (bioMerieux NASBA NucliSens assay)**
  - For the past 13 years, VRDL is one of 4 laboratories which have been certified nationally to perform quantitative HIV viral load assays in blood specimens for the NIH-sponsored "Women's Interagency HIV Study" (WIHS). During that time we have tested approximately 14,000 specimens. Viral load is the principle measure of disease progression in the subjects. The WIHS, established in 1993, is a collaborative, multi-site, natural history study designed to investigate the biological and psychological impact of HIV infection on U.S. women, and link basic science findings and laboratory techniques to the characterization of epidemiologically well-defined populations. The study population includes approximately 2000 HIV-infected women and 500 HIV-uninfected women (age 13 years or older at the time of enrollment) who are at high risk of acquiring HIV. The WIHS is comprised of six clinical sites, which are funded through cooperative agreements. Over 80 percent of the women currently enrolled in WIHS are from minority populations.

- **Surveillance Study (HIV-1 Oral fluid testing)**
  - Office of AIDS HIV Behavioral Surveillance Study: The CDC National HIV Behavioral Surveillance (NHBS) system is an ongoing project designed to collect risk behavioral data from populations at high-risk for HIV infection (men who have sex with men, injecting drug users, and high risk heterosexuals). The Office of AIDS is conducting this study in the San Diego area. Oral fluid samples are collected at a Sexually Transmitted Diseases clinic and sent to VRDL for testing. Samples are screened by an FDA-approved EIA test and those with positive results are confirmed by an FDA-approved oral fluid Western blot.

- **"Antibody Profile" test**
  - Determines patient from which sample originated by unique patient antibody reaction with cellular antigens.
  - Many samples are from CDC or referred by CDC. An example is a large epidemiologic study in which it was possible for us to rule out that discrepant test results were the result of mixing up samples from different subjects.

- **Pre-market evalulations of HIV immunoassays**
  - The Retrovirus Diagnostic Section frequently takes advantage of its large collection of archived specimens to evaluate commercial immunodiagnostic kits and related products prior to licensing. This helps to assure the public health relevance of such commercial products. Recently, diagnostic products have been evaluated in this way for HIV-1/2 IFAs, HIV-1 and HIV-2 Western Blots, and HIV-1/HIV-2 rapid tests

- **HIV-2 Western blot - New York City Department of Health**
  - To differentiate HIV-1 and HIV-2
  - Due to immigration patterns, New York City has the highest prevalence of HIV-2 in the United States. By participating in their testing algorithm, VRDL provides public health assistance while maintaining expertise with this test

- **HIV Applied Research**
  - Since the inception of the HIV pandemic, the Retrovirus Diagnostic Section has been internationally recognized for the development and application of HIV neutralizing antibody assays. This technology has been applied to studies of the natural history of HIV disease, the development of experimental HIV vaccines and the development of immunotherapeutics.
  - The major obstacle in development of both HIV vaccines and therapeutics is the presence of an extraordinary number of highly mutable viral variants. To confront this obstacle, in collaborative studies partially funded by the National Institutes of Health (NIH), both a vaccine and a passive immunotherapy are under development based on a monoclonal antibody which targets the CD4 viral receptor on the host cell -- which is shared by *all* HIV variants.
  - In collaborative NIH-supported studies, VRDL is characterizing "catalytic antibodies" which not only recognize and bind to HIV glycoproteins, but also then cleave such proteins in the manner of an enzyme. This unusual, irreversible antibody action has potential applications for diagnostics, vaccines and therapeutics.

External Funding and Grant Support

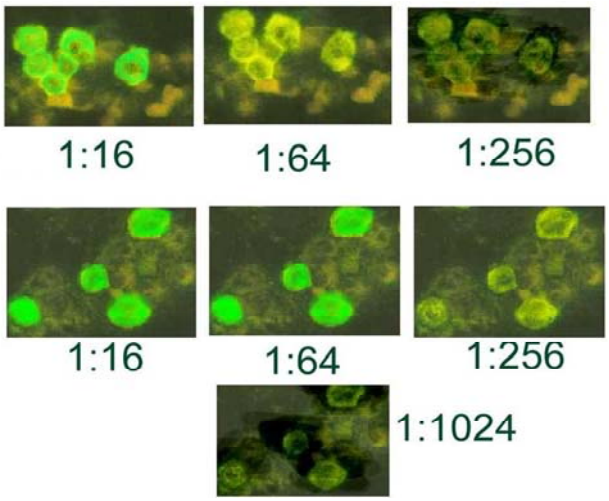
Arboviral Immunoserology: NIH AI-65359 (Project Director: Hanson), dates: 05/20/2005 - 04/30/2009: This sub-project of the Pacific Southwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, is developing serologic tests for rapidly distinguishing among infections by flaviviruses, alphaviruses and other arboviruses of potential bioterrorism importance.

Proteolytic antibody HIVcides: NIH R21 AI071951 (PI: Paul), dates: 9/15/2006 – 8/31/2008: The goal is to test the potential of catalytic antibodies which cleave HIV gp120 as topical microbicides for HIV disease.

Nucleophilic Antibodies: Characterization and Induction: NIH 1 R01 AI067020 (PI: Paul), dates: 07/01/2006-06/30/2011: The major goals of this project are to: (a) characterize the functional properties of antibodies that irreversibly bind HIV gp120 and catalyze the cleavage of this glycoprotein, (b) study the structural basis of these activities, and (c) study the neutralzing IgA responses induced by mucosal immunization with electrophilic gp120 analogs.

Figure 1. Immunofluorescence assay for antibody:
In the top three panels, a patient serum is reacted with HTLV-I, and in the next four panels with HTLV-II. Comparison of endpoint antibody titers identifies which of the two viral infections elicited the antibody response.

Sera reacting against the HTLV-I antigen in the MT-2 cell line



Sera reacting against the HTLV-II antigen in the Clone 19 cell line

Epidemiology Support Section

Section Chief: Dr. Haynes Sheppard

Background

The work of the Epidemiology Support Section (ESS) focuses mainly on HIV diagnostics in humans and research in both domestic and international arenas. In California, the section mainly provides HIV reference testing, including measuring of HIV viral loads and antiviral resistance testing, for special treatment and/or research projects implemented by the Office of AIDS and the University of California. The ESS also participates in clinical studies of HIV/AIDS vaccine candidates and pre-clinical studies of whole inactivated HIV-1 as a possible vaccine candidate. The section also performs reference testing for SNV (hantavirus) in humans, and provides support to the DCDC Vector-Borne Disease Section (VBDS) to test rodents for SNV throughout California. Other diagnostic testing includes characterization of influenza viruses for antiviral resistance. Environmental monitoring for potential agents of bioterrorism is performed through the Office of Homeland Security BioWatch program.

The section is also involved in multiple international collaborations in Africa, supporting such work as HIV vaccine development, development of low-cost assays for HIV characterization, and HIV inactivation in breast milk.

Training

For the past several years, VRDL Section Chiefs have participated as mentors for the CDC/APHL Emerging Infectious Diseases (EID) Fellowship Program. In 2007, a pre-doctoral EID fellow in the ESS section worked in collaboration with UC Davis to conduct a clinical study to validate the determination of the CD4 counts using blood from a finger-stick rather than the standard venous blood sample. This method would provide a simpler, lower cost approach to point-of-care CD4 measurements.

A second 2007/2008 postdoctoral EID fellow was assigned to ESS but will start in January 2008, and will continue the collaboration with Arcxis Biotechnologies (a spin off from the Sandia National Lab) on a project to apply Arcxis molecular detection technology to HIV for the development of simple low-cost methods for detection and quantitation of HIV. This methodology would provide infant diagnosis and viral load monitoring (to support anti-retroviral treatment) in resource-limited settings.

Laboratory Testing, Diagnostic Services and Reference Testing

- **Laboratory Support for Office of AIDS Drug Assistance Program**
  - Coordination & reference for LPHLs doing HIV viral load testing
  - Coordination & reference for LPHL and private laboratories doing antiviral resistance testing
  - Low-cost assay development and evaluation for CD4 counts and HIV viral loads [monitoring Highly Active Antiretroviral Therapy (HAART) in resource-poor settings]

- **Sin Nombre virus (SNV) testing**

- In 2007, 60 human samples were tested for possible hantavirus infection with SNV; of these, one case from El Dorado county tested positive after a commercial laboratory reported detecting IgM and IgG antibodies to hantavirus. The CDPH VRDL confirmed IgM antibodies to SNV in the acute specimen, as well as IgM and IgG antibodies in a convalescent specimen. The case-patient was an employee of the U.S. Forest Service and reported frequently entering buildings where evidence of rodents was noted. Building inspection and rodent surveillance at the patient's worksite identified potential routes of rodent ingress and/or evidence of rodent infestation (e.g., feces, chew marks) in nearly every building. A total of 25 deer mice (*Peromyscus maniculatus*) were captured; antibodies to SNV (titer = 1:400) were detected in one. CDPH recommended practicing rodent exclusion and ongoing trapping at affected buildings, as well as removal of rodent harborage around the exterior. In cases of heavy rodent infestation, VBDS recommended that staff consider using personal protective equipment before entering rodent-infested buildings or dwellings.

- In 2007, VRDL supported the surveillance and control activities of the DCDC VBDS by testing for SNV antibody in rodents. Over 1,300 rodents were tested from 16 local health departments, with 187 (12.6%) testing positive for SNV infection, including from the following jurisdictions: Alameda (0/28), Butte (2/13, 15.4%), El Dorado (37/180, 20.6%), Lassen (16/307, 5.2%), Los Angeles (0/2), Madera (11/45, 24.4%), Mariposa, (1/10, 10.0%), Mono (66/233, 28.3%), Napa (0/20), Orange (17/270, 6.3%), Riverside (4/121, 3.3%), San Bernardino (0/8), San Diego (1/113, 0.9%), Santa Barbara (15/80, 18.8%), Siskiyou (6/22, 27.3%), Tuolumne (3/25, 12.0%), and Yuba (9/129, 7.0%).

- **Sequencing of influenza isolates to assess drug resistance mutations**
  - Assess the presence of drug resistance mutations
  - Phylogenetic characterization
  - Detection of reassortants and recombinants
  - Detection of pathogenicity features
  - Developmental diagnostics, including avian influenza

- **Biowatch Laboratory**
  - Monitors the air for bioterrorism agents (funding is "in kind" from the Department of Homeland Security). Four microbiologists and one Ph.D. supervisor are supported by this program.
  - Regional Environmental Protection Agency air monitoring sites are located in the Bay Area. The samplers monitor the air 24 hours a day, 7 days a week. The VRDL BioWatch laboratory tests filters from these samplers using advanced molecular techniques that can quickly and accurately detect the presence of low levels of a bioterrorism agent's unique DNA. If and when such agents are detected, the BioWatch laboratory is responsible for initiating an appropriate response at the local, State, and Federal levels. The Bay Area BioWatch program has been enhanced recently with the addition of new automation and sample tracking systems. The number of collectors that are monitored has been more than doubled. The Bay Area BioWatch program is currently being further improved with the addition of multiplexing technology that will triple the number of assays that can be performed each day.

Special Diagnostic and Research Projects:

- **HIV Incidence Estimates using STARHS (Serologic Testing Algorithm for Recent HIV Seroconversions) Testing** (Collaboration with Office of AIDS)
  - National HIV Incidence project
  - ESS provides specimen processing for CDC STARHS testing laboratory in New York

- **Northern California Center the Biology of HIV in Minorities** (Collaboration with Universitywide AIDS Research Program)
  - Sequence analysis of HIV quasispecies from different body fluids. Studies in Minority Populations in California.
  - Immune activation during initiation of HAART

- **Laboratory Support for HIV Vaccine/Prevention Trials Networks**
  - Centralized measurement of cellular immune responses to vaccines, conducted at the Joint Clinical Research Center in Kampala Uganda

- **Inactivation of HIV in breast milk as a method to prevent mother-to-child transmission of HIV**
  - Assessment of the extent and kinetics of heat inactivation of HIV under various conditions. We have demonstrated and published that this method inactivates all detectable HIV while preserving the beneficial nutritional and immunologic properties of breast milk.
  - Recent funding at University of California Davis and University of California Berkeley for a field trial of feasibility in Africa.

- **Prevention of mother-to-child transmission (PMTCT)** (Collaboration with Global Strategies for HIV prevention)
  - Workshops in prevention of mother-to-child HIV transmission and other prevention methods.
  - Rapid HIV testing & low-cost treatment monitoring

- **AIDS Vaccine Development**
  - Pre-clinical studies of whole inactivated HIV as a vaccine candidate
  - Bioreactor production – Psoralen + Aldrithiol-2 inactivation

External Funding and Grant Support

AIDS Vaccine Development: NIH 1R21AI073236-01A1 (PI: HW Sheppard), start date: 6/1/07. Preclinical studies of whole Inactivated HIV-1 as a vaccine candidate.

Laboratory Support for HIV Vaccine/Prevention Trials Networks: Pediatric HIV vaccine trial in Uganda. NIH U01 AI068632 (PI: Brooks Jackson, John Hopkins University).

Northern California Center the Biology of HIV in Minorities: UARP grant CH05-D-606 (PI: Rich Pollard, UC Davis).

Inactivation of HIV in breast milk as a method to prevent mother-to-child transmission of HIV: Thrasher foundation grant to UC Berkeley, award # 02819-4 (PI: Kiersten Israel-Ballard) and NIH R21 HD051473 (PI: Caroline Chantry, UC Davis).

Medical & Epidemiology Liaison Section

Section Chief: Dr. Janice Louie

Background
The Medical and Epidemiology Liaison Section works to support the diagnostic services of the above sections with regard to coordination of diagnostic testing (e.g., medical screening, record-keeping, biosafety, and adherence to CLIA and HIPAA standards), as well as to organize and manage specific surveillance and training activities.

Local Assistance Unit
The functions of the Local Assistance Unit are to provide technical consultation to other laboratories and programs concerning the proper collection and shipment of appropriate clinical specimens for virology testing and to assist with the interpretation of laboratory results. In addition, the Local Assistance Unit oversees the processing of clinical specimens, including: receives all virology diagnostic samples and assures that the information on the samples matches the submittal forms, enters patient demographic and sample data into the Laboratory Information System (LIMS) database, routes samples for diagnostic testing, and generates and distributes reports back to the submitter and the local health officer. This Unit also includes the VRDL CLIA Quality Control Officer, who works with staff to oversee the Quality Assurance procedures and extensively review VRDL test offerings and protocols. The Local Assistance Unit is also responsible for development and implementation of LIMS into VRDL.

The Local Assistance Unit includes the MDL/VRDL Training Officer and is responsible for conducting both formal training for the Public Health Microbiologist certification as well as in-house training. In 2007 and 2008, VRDL was unable to provide the one month training in virology required for certification of Public Health Microbiologist trainees, due to staffing limitations. The VRDL was able to provide some lecturers and administrative assistance to staff from Marin County Public Health Laboratory who provided this training for fifteen trainees. The Local Assistance Unit is also responsible for in-house training of microbiologists and other staff members for both the VRDL and the MDL laboratories. Training activities include Microbiologist Seminars held approximately monthly and American Society of Microbiology and APHL teleconferences. The Local Assistance Unit represents the VRDL on the LPHL Directors training committee and the LPHL Annual Institute planning committee.

Epidemiology Liaison Unit
The role of the Epidemiology Liaison Unit is to oversee, coordinate and/or assist with a diverse program of laboratory-based surveillance activities. Long-term surveillance activities include encephalitis, WNV, influenza, acute viral gastroenteritis (e.g., norovirus) and unexplained deaths due to infectious etiology. A summary of the major surveillance activities, organized by syndrome or diseases of public health importance, are provided in the following pages of this report.

External Funding and Grant Support

Epidemiology and Laboratory Capacity for Infectious Diseases: National Center for Infectious Diseases (NCID) Grant Number: U50/CCU923677 (PI: D. Schnurr).

CDC Emerging Infections Program: Grant Number: U50/CCU915545 (PIs: A. Reingold, D. Vugia).

Laboratory-Based Surveillance Projects

- **California Encephalitis Project**
- **California Influenza Surveillance Project**
- **California Rabies Surveillance Program**
- **Unexplained Death Project**
- **Viral Gastrointesteritis (Norovirus) Outbreaks**
- **West Nile Virus Surveillance**

California Encephalitis Project

This report summarizes the activities of the California Encephalitis Project (CEP). CEP is the largest prospective study of encephalitis of its kind in the world. To date, over 3,500 persons with suspected encephalitis have been referred to the CEP.

1. Case ascertainment and enrollment:

Encephalitis is a complex syndrome and the etiology is often not identified. CEP was initiated in 1998 to identify the causes and further describe the clinical and epidemiologic features of encephalitis. Physicians from more than 220 facilities (including coroner's offices) in California have referred cases to the CEP. A standardized report form is used to collect demographic and clinical data. Serum, cerebrospinal fluid (CSF), and respiratory samples are obtained prospectively and tested for 16 potential infectious causes of encephalitis (see Table 1). The link between an identified infection and encephalitis is defined using predetermined, organism-specific criteria for confirmed, probable, or possible causes (Glaser et al, 2003).

Table 1: Listing of core testing, 1998-2007

Specimen Type	Agent	Test type	Comments
CSF	Herpes consensus (HSV -1 and -2, VZV, CMV, EBV, HHV-6)	PCR	Discontinued in 2003 (changed to individual herpes PCR)
	HSV-1	PCR	Initiated in 2003
	HSV-2	PCR	Initiated in 2003
	VZV	PCR	Initiated in 2003
	HHV-6	PCR	Initiated in 2006
	Mycoplasma pneumoniae	PCR	Initiated in 1999 (performed for cases with evidence of acute infection, e.g. positive serology)
	Enterovirus	PCR	
	Measles	Serology	
	General viral isolation	Culture	Discontinued in 2000 (due to low yield)
Acute serum	St. Louis encephalitis	Serology	
	Western equine encephalitis	Serology	
	West Nile virus	Serology	Initiated in 2001
	EBV	Serology	
	Enterovirus	Serology	Discontinued in 2003 (no longer part of core testing; testing performed for clinically compatible cases only)
	Mycoplasma pneumoniae	Serology	
	Measles	Serology	
	Bartonella spp.	Serology	1998-2003 (no longer part of core testing)
Convalescent serum	St. Louie encephalitis	Serology	
	Western equine encephalitis	Serology	
	West Nile virus	Serology	Initiated in 2001
	Mycoplasma pneumoniae	Serology	
	HSV	Serology	
	VZV	Serology	
	Adenovirus	Serology	
	Chlamydia spp.	Serology	
	Influenza A and B	Serology	Testing performed during respiratory season
	Bartonella spp.	Serology	Discontinued in 2003 (no longer part of core testing; testing performed for clinically compatible cases only)
Respiratory sample	Enterovirus	PCR	Initiated in 2003
	Mycoplasma pneumoniae	PCR	Initiated in 2001
	Influenza A and B	PCR	2003-2004 (changed to full respiratory panel)
	Respiratory panel: Influenza A and B, adenovirus, human metapneumovirus, RSV type A, parainfluenza types 1-4	PCR	Initiated in 2004 (performed on specimens for patients with upper respiratory infections)
	General viral isolation	Culture	

Abbreviations: (HSV) herpes simplex virus; (VZV) varicella zoster virus; (CMV) cytomegalovirus; (EBV) Epstein-Barr virus; (HHV-6) human herpesvirus-6; (PCR) polymerase chain reaction; (spp) species; (RSV) respiratory synctial virus.

Further testing is based on patient exposure information, travel history, and clinical information [e.g. Rabies, *Bartonella spp.*, *Mycobacterium tuberculosis*, *Balamuthia mandrillaris*]. When testing is not available at VRDL, specimens are sent to an appropriate reference laboratory [e.g. UC Davis (*Coccidioides immitis*), Purdue University (*Baylisascaris procynosis*)].

The CEP collaborates with Emerging Infection Program sites in Tennessee and New York on various aspects of encephalitis including establishment of a more specific case definition, modification of the case history form to enhance data collection, and expansion of testing algorithms. Additionally, data are shared between the three sites and CDC informally by e-mail or more formally on teleconferences.

2. Results:

Agent-specific findings:

CEP has made progress towards identifying particular agents linked to encephalitis.

Agent	CEP findings/comments
Balamuthia mandrillaris	In 2007, a MMWR report was prepared describing cases of *Balamuthia mandrillaris* identified in the CEP. Out of a total of >2,000 encephalitis cases submitted to the CEP, ~300 have been examined for balamuthiasis. CEP testing has helped to diagnose 10 cases. All patients were immunocompetent; 8 were Hispanic-Americans; 5 were children. The cases that were detected through CEP testing would have otherwise been missed. It is likely that other cases occur but are not diagnosed because of lack of familiarity with the disease.
Herpes simplex virus - 1 (HSV-1)	HSV PCR remains a sensitive method for the rapid detection of encephalitis due to HSV, but there may be important limitations. Twenty percent of our cases were negative for HSV by PCR on initial lumbar puncture. In these cases, serologic testing of sera and CSF served as a useful adjunctive tool in confirming the diagnosis of HSE. For patients whose clinical presentation is compatible with HSE, in the setting of a negative CSF HSV PCR early in the clinical course, CSF and paired sera antibody testing should be utilized to complement PCR in establishing a diagnosis. A poster was presented at the 23rd Clinical Virology Symposium in May 2007.
Mycobacterium tuberculosis (MTB)	Many patients with MTB in the CEP presented with atypical clinical features and in most cases MTB was not suspected at the time of referral. A report summarizing the clinical and laboratory features of 19 laboratory confirmed MTB cases and a discussion on testing strategies has been published (Christie, 2008).
Mycoplasma pneumoniae	Since Fall of 2006, the CEP has been collaborating with Joel Baseman of the University of Texas to determine if *Mycoplasma pneumoniae* infection is toxin mediated. Since 1998, Mycoplasma is the most common agent identified in specimens of patients referred to the CEP. However, the role of Mycoplasma as a cause of encephalitis is still unclear. A manuscript has been published (Christie, 2007).
Parvovirus B-19	CEP initiated Parvovirus B-19 testing in 2006 to further evaluate its role in encephalitis. Among the 441 acute sera referred to CEP, 7 (1.5%) were Parvovirus B-19 IgM (+). Three patients were ≤18 years; 4 were female and 3 were Hispanic. None of the Parvovirus B-19 IgM (+) cases had a URI prodrome or a rash. Also, 3 of the patients had a co-infection: 2 with possible *Mycoplasma pneumoniae* ; 1 with confirmed HSV 1. Of the 4 available CSF samples, 1 was Parvovirus B-19 (+) by PCR. A poster was presented at the IDSA 45th Annual Meeting in October 2007.
Enterovirus	CEP and CDC have been collaborating to describe the clinical and laboratory features of enterovirus encephalitis. Additionally, we will focus on differences between the strain types. A report of these findings has been published (Fowlkes, 2008).
Measles subacute sclerosing panencephalitis (SSPE)	In 2004, the CEP published a report describing five cases of measles SSPE that emphasized the importance of considering SSPE in the differential diagnosis of encephalitis, particularly among pediatric patients (Honarmand, 2004). SSPE was not suspected in the differential diagnosis of three of the cases until CEP measles testing was done. The diagnosis of SSPE is often not considered by clinicians due to it's rarity in the U.S. and the nonspecific clinical manifestations at onset.

[1] Christie LJ et al. Diagnostic challenges of central nervous system tuberculosis. Emerg Infect Dis 2008; 14(9):1473-5.
[2] Christie LJ et al. Pediatric encephalitis: What is the role of Mycoplasma pneumoniae? Pediatrics 2007; 120:305-13.
[3] Fowlkes AL et al. Enterovirus-associated encephalitis in the California encephalitis project, 1998-2005. J Infect Dis 2008;198(11)1685-91.
[4] Honarmand S et al. Subacute sclerosing panencephalitis in the differential diagnosis of encephalitis. Neurology 2004; 63(8):1489-93.

Clinical Profile Analysis

Despite the enrolled patients meeting a common case definition for encephalitis, there is tremendous variability in the clinical presentation, hospital course, laboratory features and outcomes. Recognizing that encephalitis is a heterogeneous syndrome, to further evaluate the syndrome, ten distinct constellations of clinical features, 4 focal and 6 generalized clinical profiles, were recognized in approximately one-half of the cases referred to the CEP (see Table 2). Cases within a given profile are further analyzed to determine if they have other common features such as prodromal symptoms, course of the illness, demographic characteristics, infectious or non-infectious causes, and/or outcomes. The recognition of discrete clinical profiles among encephalitis patients should help focus our efforts to understand the etiology, pathogenesis, course of illness and management of this complex syndrome. The following is a listing of the profiles along with a short description:

Table 2. Clinical profiles of CEP cases, 1998-2007

	Description
Focal	
Temporal Lobe	Patients with temporal lobe enhancement on neuroimaging. Patients with temporal lobe activity on EEG only (i.e. no temporal lobe enhancement of MRI or CT)
Extrapyramidal (movement disorder)	Patients presenting with movement disorders (presumably related to extrapyramidal system).
Cerebellar	Patients presenting with a predominance of cerebellar signs (ataxia, dysarthria, dysmetria) and/or focal cerebellar lesions on MRI.
Hydrocephalus	Patients presenting with hydrocephalus on MRI
Generalized	
Diffuse cerebral edema	Patients presenting with diffuse cerebral edema (as evidenced by neuroimaging or autopsy)
Intractable seizures (treated with anesthetic coma)	Patients presenting with or developing intractable seizures requiring anesthetic coma for management.
Primarily seizure with rapid recovery	Patients presenting predominantly with seizures and recovery occurring within 7 days.
Psychosis presentation	Patients with psychosis at admission (day hospitalized)
Dementia presentation	Patients with dementia at admission
Recurrent or Chronic inflammatory CNS disease	Patients presenting with recurrent or chronic CNS disease
Muti-focal white matter lesions	Patients presenting with multifocal white matter involvement on neuroimaging.

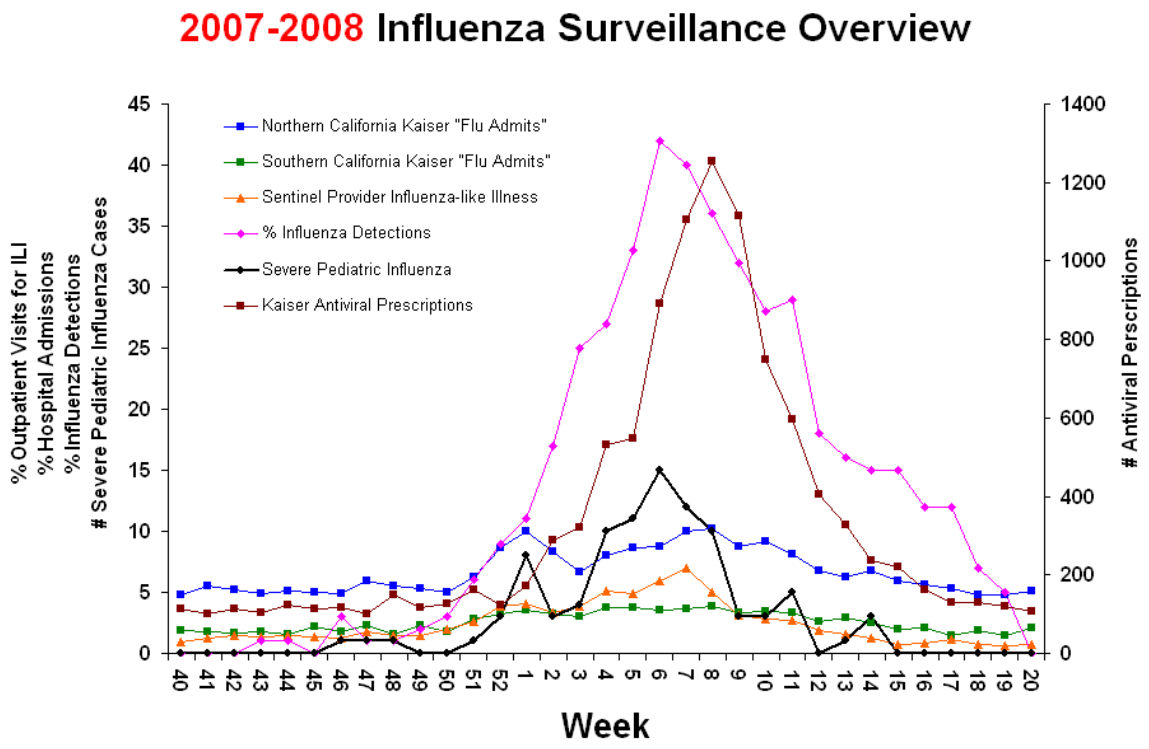
Table 3. Confirmed/Probable Causes of Encephalitis Identified, California, 1998-2007

Enterovirus	82
HSV - 1	55
VZV	35
West Nile virus	31
EBV	26
MTB	25
HHV6	20
CJD	18
Bartonella-CSD	15
Balamuthia mandrillaris	8
HSV - 2	7
Measles SSPE	6
Hepatitis C	5
Rabies	5
Coccidioidomycosis	4
HSV1/ HHV6	4
pyogenic bacteria	4
Acute HIV	3
Baylisascaris Procyonis	3
RMSF	3
Mycoplasma pneumoniae	2
Rotavirus	2
Adenovirus	1
Angiostrongylus	1
Cryptococcus	1
Gnathostomiasis	1
Q Fever Phase II	1
Tropheryma whippelii	1

California Influenza Surveillance Project 2007-08 Influenza Season

Note: this summary includes 2008 data given that influenza activity is monitored over the winter season, which typically begins in October and ends in May of the subsequent year

Overall the influenza season was moderate in severity, with high levels of activity in January, February and early March. The magnitude of influenza activity as described by the multiple parameters measured (percentages of Kaiser Permanente inpatient admissions for influenza and pneumonia, Kaiser Permanente antiviral prescriptions, CDC sentinel provider outpatient visits for influenza-like illnesses (ILI), laboratory detections for influenza, and severe pediatric influenza illnesses) was comparable to previous years, roughly peaking during weeks 2-12 of the influenza season.



Highlights from the 2007-08 season include:

- **Influenza-associated pediatric deaths:**

This season 6 fatal cases of pediatric influenza were reported. This is comparable to the number of fatal cases in previous years: 6 (2006-07), 13 (2005-06), 5 (2004-05) and 8 (2003-04). This season two of the deaths were due to influenza A and four due to influenza B. Two cases presented with severe encephalitis. No fatal cases had secondary bacterial infection. Only one case had been vaccinated for influenza.

- **Strain typing and vaccine mismatch**

Over 1008 respiratory specimens were tested at VRDL this season; 409 were positive for influenza, including 288 (70%) influenza A and 121 (30%) influenza B. As the season evolved laboratory strain typing data began to show that there was a vaccine mismatch occurring between two of the three components of the influenza vaccine: the H3 subtype and the B subtype (the H1 subtype still matched fairly well). Interim results from a study carried out in Wisconsin found vaccine effectiveness of 58% against circulating influenza A/H3 viruses, based on data collected from Jan 21 through Feb 8, 2008. No vaccine effectiveness against influenza B viruses was found. The new strains circulating have been identified as A/Brisbane/10/2007-like and B/Florida/04/2006-like by both CDC and VRDL. Both these new strains will be included in the 2008-09 influenza vaccine.

- **Antiviral resistance testing**

Two drugs are available to treat influenza: amantadine and oseltamivir. Antiviral resistance monitoring was instituted at VRDL following the emergence in 2005-06 of new drug resistance to amantadine, a drug that was previously commonly used for both treatment and prophylaxis of influenza. In addition, both at CDC and VRDL, this season the emergence of resistance to oseltamivir was identified. This season, 157 influenza-confirmed specimens were tested for antiviral resistance. 100% (87/87) of influenza A/H3 viruses and 4% (3/70) of influenza A/H1 viruses had the S31N mutation consistent with adamantane resistance. No mutations consistent with resistance to the neuraminidase inhibitors were seen in A/H3 viruses (0/87). Ten percent (7/70) of A/H1 viruses had the H274Y mutation associated with oseltamivir resistance.

A breakdown of how the individual parameters are measured is described below:

Kaiser Permanente Inpatient Data

"Flu admits" were defined as inpatient hospital admissions for the diagnoses of pneumonia or influenza. ICD-9 discharge codes 480-487 are well known to correlate with influenza activity but are not useful for tracking activity in real time. Based on data collected in previous years, admitting diagnoses of "flu", 'influenza" and "pneumonia" approximate ICD-9 codes 480-487, and were used to track influenza activity. "Flu admits" are present year-round because of baseline pneumonia admissions. The estimated baseline rate for the off-season is approximately 3-5%. The percentage of "Flu admits" was calculated by dividing the number of flu admissions by the total number of hospital admissions for the same day. Admissions for pregnancy, labor and delivery, birth, and same day surgery procedures were excluded from the denominator.

Kaiser Permanente Antiviral Usage Data

The number of prescriptions filled for the antiviral drugs used to treat influenza (amantadine, rimantadine, zanamivir and oseltamivir) by Kaiser outpatient pharmacies in California is reported to us weekly. Baseline amantadine usage is present year-round for disorders such as Parkinson's disease. Because of reports of widespread resistance to the adamantine drugs, oseltamivir was the main drug prescribed for treatment of influenza in the 2007-08 season.

Sentinel Physician Influenza-Like Illness Data

Over 150 sentinel providers located throughout California participate in the CDC Sentinel Provider Influenza Surveillance Program. These sentinel providers report weekly data on the percentage of outpatient visits seen for influenza-like illness, calculated by dividing the number of influenza-like illness visits by the total number of outpatient visits per week. Influenza-like illness is defined as fever (> 100° F [37.8° C], oral or equivalent) AND cough and /or sore throat (in the absence of a KNOWN cause other than influenza). One hundred fourteen providers were "active", reporting ILI more than 50% (16 weeks) of the time during the 2007-08 influenza season.

Severe pediatric influenza and pediatric influenza –associated deaths

Local health departments work with infection control practitioners at the 26 hospitals in California with a pediatric intensive care unit (PICU) to encourage reporting of cases and submission of specimens from patients that meet the following criteria: 1) age 0-17 years; 2) laboratory-confirmed influenza; and 3) hospitalized in the PICU or expired at any location (e.g. hospital, emergency room, home, etc). Data on cases that expire at home and are not evaluated in the hospital setting are captured by local health department medical examiners, who are asked to report pediatric fatalities that are laboratory-confirmed for influenza either prior to death or at autopsy. Demographic and clinical data is collected by local health departments and reported by fax to CDPH.

Respiratory Virus Isolation/Detection Data

During the 2007-2008 influenza season, CISP received weekly reports of laboratory detections and isolations of influenza and other respiratory viruses (predominantly RSV) from 22 participating sites situated throughout California, including hospital, academic, public health, and private laboratories. The CDPH VRDL also encouraged submission of clinical respiratory specimens and isolates from a wide variety of settings, including LPHL and clinical laboratory partners, hospitalized cases of severe respiratory illness, outpatient clinics and outbreak settings. Selected isolates were forwarded to CDC for confirmation and further analysis

In the 2007-08 season, a total of 1008 clinical specimens were tested at VRDL using R-mix shell vial testing and viral isolation in primary monkey kidney and human fetal diploid cells; 505 (50%) had positive yield by isolation. 409 isolates were positive for influenza: a majority (287/409; 70%) were identified as influenza A (91 H1 and196 H3) compared to influenza B (134/409; 33%). These results are comparable to those reported by the World Health Organization National Respiratory and Enteric Virus Surveillance System (NREVSS) laboratory network, where across the US, of the 39,453 influenza viruses isolated, 28,105 (71.2%) were influenza A viruses and 11,348 (28.8%) were influenza B viruses. Ninety-six of the 505 isolates had non-influenza viral pathogens diagnosed, including rhinovirus (34); parainfluenza (26); adenovirus (20), RSV (4), coxsackievirus (4); echovirus (4); herpes (3); human metapneumovirus (1).

Two hundred and seventy one influenza isolates (65 H1, 79 H3 and 127 B) underwent detailed antigenic characterization by the haemagglutination inhibition assay (HIA).

Subtype A/H1

Fifty-two (80%) A/H1isolates were characterized as A/Solomon Islands/3/6-like and 13 (20%) A/H1 isolates showed reduced HIA titers (low reactors) in our test with post infection ferret antisera produced against A/Solomon Islands/3/6-like (H1N1).

A/Solomon Islands/3/6-like was recommended as the H1 component of the 2007-08-influenza vaccine for the Northern Hemisphere.

Subtype A/H3

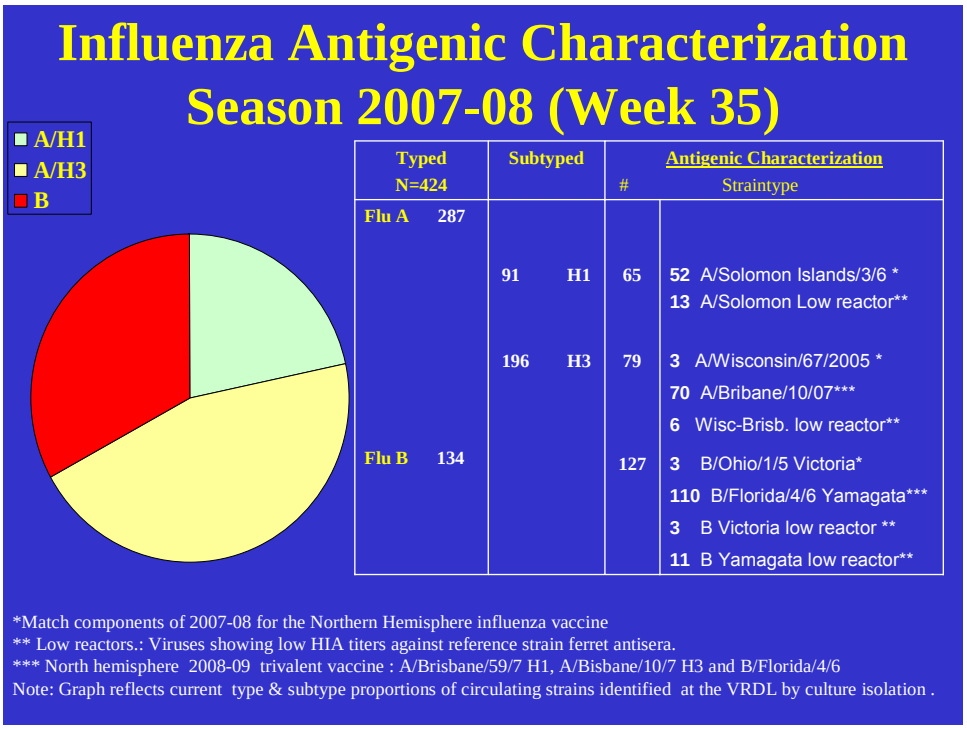
Seventy (88%) A/H3 isolates were characterized as A/Brisbane/10/07-likeand 3 (4%) A/H3 isolates were characterized as A/Wisconsin/67/2005-like;6 (8%) A/H3 isolates showed reduced HIA titers (low reactors).

A/Wisconsin/67/2005-like was recommended as the H3 component of the 2007-08-influenza vaccine for the Northern Hemisphere.

Type B

Among the B isolates 3 (3%) were characterized as B/Ohio/1/5-like (Victoria-lineage). One hundred and ten (86%) B isolates were characterized as B/ Florida/4/6-like belonging to Yamagata-lineage. Fourteen (14%) B isolates showed reduced HIA titers (low reactors) with post infection ferret antisera against B/ Florida/4/6 and B/Ohio/1/5, 11 and 3 respectively.

B/Ohio/1/5-like (Victoria-lineage) was the B component of the North Hemisphere 2007-08 influenza vaccine.



The WHO recommended components for the 2008-2009 influenza vaccine for the Northern Hemisphere are:

- For the H1N1 component, a strain similar to A/Brisbane/59/2007, replacing A/Solomon Islands/3/2006

- For the H3N2 component, a strain similar to A/Brisbane/10/2007, replacing A/Wisconsin/67/2005
- For the B component, a strain similar to B/Florida/4/2006, replacing B/Malaysia 2506/2004

Disclaimer: *It is important to note that our surveillance system does not receive data from ALL labs, physicians, hospitals, or pharmacies in California; therefore our numbers reported do not represent all cases of influenza, but are intended to demonstrate trends in influenza activity.*

More graphs and seasonal summaries can be found at the California Influenza Surveillance Project website at:
http://www.cdph.ca.gov/programs/vrdl/Pages/CaliforniaInfluenzaSurveillanceProject.aspx

For further information about the California Influenza Surveillance Project, contact:

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California Rabies Surveillance Program

To better assess the risks of rabies exposure to the public, the VRDL is improving our understanding of the transmission patterns of rabies in California and neighboring states by expanding our rabies strain typing and bat species identification programs. One goal toward this effort is to develop a California Rabies Strain Gene Bank to store sequence typing data (and, potentially, other data such as monoclonal antibody panels and restriction enzyme patterns) for rabies isolates throughout the state as well as annotated sequences representing global variation of rabies isolates that are available on public databases. This database will allow comprehensive comparative analyses when characterizing unknown samples. In addition, VRDL will archive rabies-positive samples submitted by local and county public health laboratories.

To begin development of the Rabies Gene Bank, VRDL is strain typing archived rabies samples that had been submitted in previous years; however, to ensure that the database best represents the genetic diversity of rabies viruses statewide, VRDL has encouraged local and county labs interested in collaborating to submit all rabies-positive samples to VRDL for strain characterization. To best understand the roles of different bat species as reservoirs or vectors for rabies viruses in California, VRDL is also amassing a large database of bats with species identification and associated epidemiological data for both rabies-positive and rabies–negative bats submitted to VRDL from local and county laboratories. In as timely a manner as possible, VRDL supplies the strain typing information for any rabies-positive species and species identification for bats to the submitting laboratory.

The project began in late spring of 2006 and by the year end, VRDL had reported out the species identification for 1621 bats tested for rabies in 33 of the 38 county laboratories (representing 49 of the 58 counties) in California. Also in that year, the VRDL identified the rabies virus strain type for 16 rabies-positive terrestrial mammals and 38 insectivorous bats submitted. The number of bat submissions for species identification reached 1685 bats in 2007, and interest in strain typing increased to 117 strain typing requests (23 terrestrial mammals and 94 insectivorous bats) from the counties.

Rabies Trends in 2007

Rabies surveillance in 2007 was an average year for submissions with 6,799 animals tested and 189 animals positive for rabies. Since the eradication of the domestic dog-associated rabies virus variant in California in the mid-1900s, the primary reservoirs for rabies virus are striped skunks (*Mephitis mephitis*) and several species of insectivorous bats. Consistent with recent trends, in 2007, the highest incidence of rabies occurred in the insectivorous bats (153 rabies positive bats out of 1671 tested) and skunks (26 rabies positive skunks out of 652 tested). A more than decade-long trend indicating decreased incidence of rabies among skunks has continued into 2007 (Figure 1). This decrease appears to be indicative of a true decrease in rabies among skunk populations although a decrease in surveillance and testing of skunks for rabies might also be contributing to the trend.

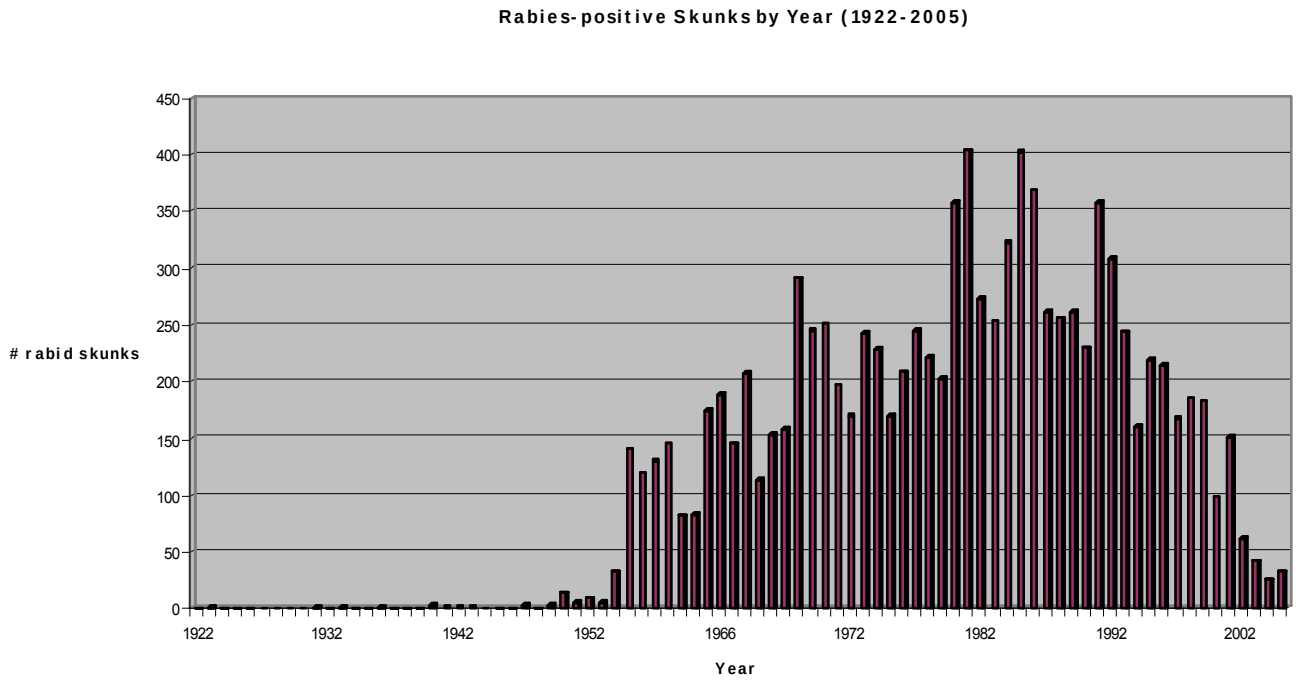


Figure 1. Skunk rabies in California over 100 years. Recent trends suggest that skunk rabies in California is at historic lows.

Identification of bat species in California

In 2006 when VRDL reinstituted bat species identification, we received and provided species identifications for 1621 bats submitted by local and county public health laboratories (representing bats tested from 2003-2006); in 2007, VRDL identified the species for 1685 bats. The most common bat species identified in California in the since 2003 include *Tadarida brasiliensis* (623 submitted in 2006; 584 in 2007), *Myotis californicus* (247 submitted in 2006; 255 in 2007), *Myotis yumanensis* (199 submitted in 2006; 219 in 2007), *Eptesicus fuscus* (180 submitted in 2006; 121 in 2007), and *Lasiurus cinereus* (62 submitted in 2006; 53 in 2007).

The numbers presented in Table 1, which shows the percentage of rabies-positive bats by species, reflect a biased sampling of moribund and dead individuals and should not be interpreted as the true prevalence of rabies in these bat species. In addition, not all counties submitted all bats tested (i.e., some counties submitted only rabies-positive or rabies-negative bats) further contributing to biases in prevalence estimates. Scientific studies have established that the prevalence of rabies in bat populations sampled randomly is approximately 0.5-2.0%. In addition, the high percentage of rabies-positive individuals found in some species (e.g., Silver-haired bats [*Lasionycteris noctivagans*], Yellow bats [*Lasiurus xanthinus*], long-legged Myotis [*Myotis volans*], and Townsend's big-eared bat [*Corynorhinus townsendii*] between 2006 and 2007) are likely biased because of small sample sizes. The insectivorous bat species that historically have been associated with human rabies cases in California are Silver-haired bats, *Lasionycteris noctivagans* and Mexican free-tailed bats, *Tadarida brasiliensis*.

Table 1. Rabies-positive bats by species, 2006 and 2007.

Species	2006*	% (+) 2006	2007*	% (+) 2007
Tadarida brasiliensis	623	22.4	584	11.1
Myotis californicus	247	2.6	255	3.5
Myotis yumanensis	199	4.3	219	3.2
Eptesicus fuscus	180	13.3	121	20.7
Lasiurus cinereus	62	10.2	53	7.5
Pipistrellus hesperus	46	20.9	70	18.6
Myotis ciliolabrum	43	0.0	41	0.0
Antrozous pallidus	42	5.0	70	2.9
Myotis lucifugus	28	4.2	30	3.3
Lasiurus blossevillii	27	4.3	17	0.0
Myotis evotis	22	4.8	20	9.1
Lasionycteris noctivagans	20	29.4	22	0.0
Lasiurus xanthinus	19	26.3	12	7.7
Myotis thysanodes	11	0.0	6	0.0
Corynorhinus townsendii	6	0.0	6	14.3
Eumops perotis	5	0.0	5	0.0
Myotis volans	4	25.0	12	0.0
Nyctinomops macrotis	1	0.0	0	0.0

* Numbers of bats submitted in these years include some bats that were tested in previous years (2003 through 2007).

Strain typing effort

In addition to providing species identification for insectivorous bats, the VRDL also provides strain typing information for rabies-positive animals submitted by the local and county laboratories. Strain typing information is derived using a standard protocol provided by the CDC in which a small segment of the nucleoprotein gene is sequenced and compared phylogenetically to a diverse database of known rabies virus variants collected globally. Strain typing is a critical tool used to track the movement of rabies virus variants within and between reservoir and vector species and to identify the source of rabies virus infection in humans and other non-reservoir species (e.g., domestic and peridomestic animals).

In 2006, the VRDL received requests to strain type 54 rabies-positive animals (16 terrestrial mammals and 38 insectivorous bats; See Table 2). In 2007, 117 individuals

were strained typed, including 23 terrestrial mammals and 94 bats (Table 3). These data enable the public health community to monitor for potential introductions of novel rabies virus variants or variants not previously recorded in California. These molecular epidemiological data also have the potential to identify new reservoir species, as was demonstrated rabies epizootic in the eastern United States after the introduction of the raccoon-associated rabies virus variant in the 1980s.

Table 2. 2006 rabies virus strain typing information for terrestrial mammals and insectivorous bats.

	Strain Type								
Species	Tb	Ef	Ph	Mca	My	Lc	CA skunk	Total	
Tadarida brasiliensis (Tb)	20	1		1		1		23	38 bats
Eptesicus fuscus (Ef)	6	3						9	
Pipistrellus Hesperus (Ph)			1					1	
Myotis californicus (Mca)				2				2	
Myotis yumanensis (My)	1				1			2	
Lasiurus cinereus (Lc)						1		1	
Skunk							13	13	16 terrestrial mammals
Raccoon								0	
Coyote								0	
Fox				1	1		1	3	
Dog								0	
Cat								0	
Total	27	4	1	4	2	2	14	54	

The 2006 strain typing data indicate that the California skunk rabies virus variant is the most common variant seen in rabies-positive terrestrial mammals, although, occasionally, terrestrial mammals (including humans) are infected with insectivorous bat-associated rabies virus variants. Not surprisingly, all of the rabies-positive insectivorous bats were infected with bat-associated rabies viruses. Additionally, the strain typing information provided by the nucleoprotein sequence data is sufficiently detailed to enable discrimination of different rabies virus variants associated with different species of insectivorous bats. These data have been used in the past to identify the specific rabies virus variant associated with cryptic human rabies cases in California.

Similarly, in 2007, the California skunk rabies virus variant predominated among terrestrial mammals with a few notable exceptions. First, one striped skunk was infected with a bat rabies virus variant (associated with *Myotis californicus*) and another striped skunk imported from Baja California, Mexico was infected with the Baja California skunk rabies virus variant. Additionally, both rabid raccoons detected in 2007 were infected with insectivorous bat rabies viruses as were 2 of 3 foxes and the only cat diagnosed with rabies.

Table 3. 2007 rabies virus strain typing information for terrestrial mammals and insectivorous bats.

	Strain Type											
Species	Tb	Ef	Ph	Mca	My	Lc	Lx	Ln	CA skunk	Baja skunk	Total	
Tadarida brasiliensis (Tb)	45										45	94 bats
Eptesicus fuscus (Ef)	1	21					1				23	
Pipistrellus Hesperus (Ph)			5								5	
Myotis californicus (Mca)				7		1					8	
Myotis yumanensis (My)				1	3	1		1			6	
Lasiurus cinereus (Lc)						2					2	
Lasiurus xanthinus (Lx)		1									1	
Lasionycteris noctivagans (Ln)											0	
Antrozous pallidus (Ap)	1										1	
Bat ?		2	1								3	
Skunk				1					13	1	15	23 terrestrial mammals
Raccoon	1							1			2	
Coyote									1		1	
Fox				2					1		3	
Dog									1		1	
Cat				1							1	
Total	48	24	6	12	3	4	1	2	16	1	117	

As the database of known rabies virus strains grows and genetic diversity of these viruses catalogued, more detailed epidemiological studies are possible. For example, with increasing sequence data of the California skunk rabies virus variant, evidence of geographical discrimination of rabies virus variants is increasingly apparent (Figures 2 & 3). These data can be used to track individual epizootics of skunk rabies, monitor the spread of skunk rabies into new counties, as well as to assist in monitoring rabies activity in the event that skunk rabies eradication programs are implemented in the future.

Figure 2. A phylogenetic tree representing multiple genetically differentiable lineages of rabies virus variants in skunks in California.

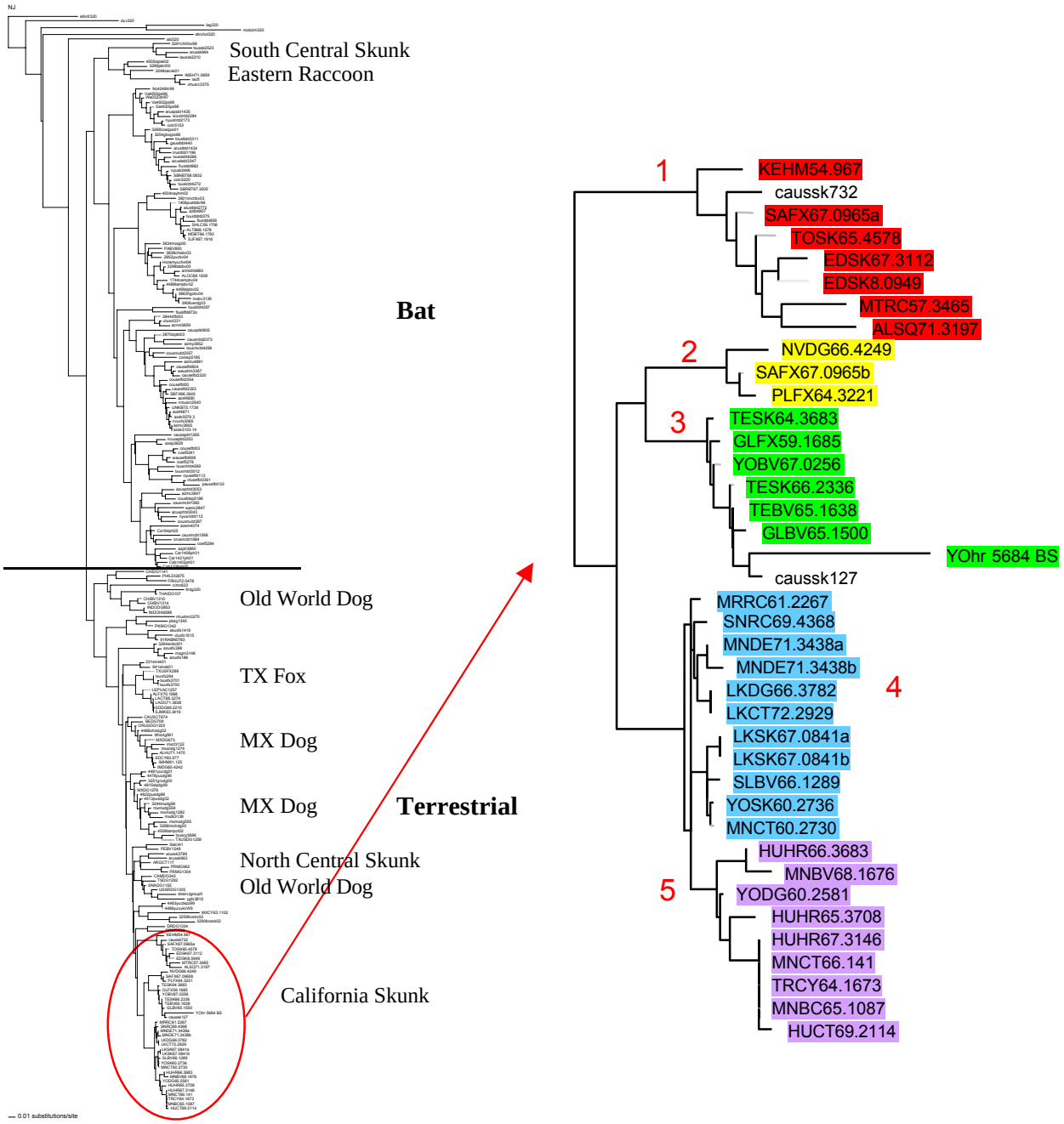
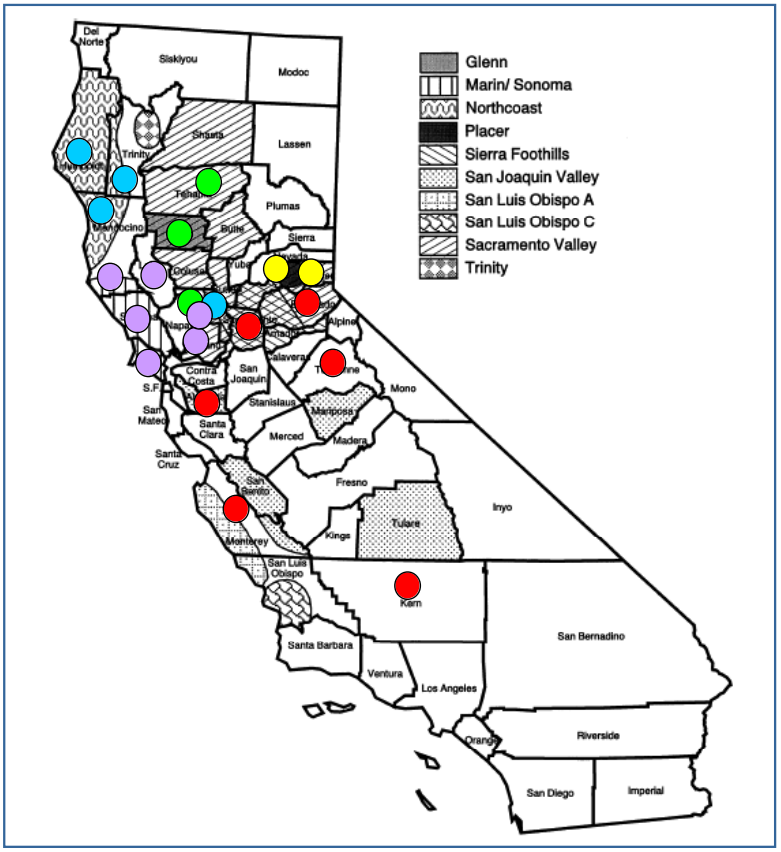


Figure 3. Individual genetic lineages of the California skunk rabies virus tend to be associated with distinct geographical regions. These sequence data agree with earlier VRDL studies using restriction enzyme analysis of California skunk rabies viruses.

Skunk Rabies Viruses in California



- = San Joaquin Valley/ Sierra Foothills
- = Placer
- = Sacramento Valley
- = North Coast
- = Marin/Sonoma

Disclaimer: *The data collected through the California Rabies Surveillance Program are based upon voluntary participation and submission of samples from the local and county public health laboratories and, therefore, represent incomplete data for the state of California. Interpretations from these data must take into account the inherent biases of such a passive surveillance program.*

For further information about the California Rabies Surveillance Program, contact:

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Unexplained Death Project (UNEX): 2007

Surveillance for infectious disease-related deaths builds critical infrastructure to detect infections of public health importance, including bioterrorism, reportable diseases, vaccine-preventable diseases and emerging infections that may be missed by traditional public health surveillance. Medical examiners investigate about 20% of all deaths in the US and have the legal authority to investigate sudden, unattended and unexplained deaths, some of which may be due to infections of public health importance. However, because of limited resources and a focus on investigating intentional or accidental deaths, autopsies may be inconsistently performed on possible infectious disease-related deaths and when they are performed, investigation may not be geared toward identification of specific etiologic agents. This may limit the capacity to recognize and report possible infection-related fatalities that may have significant implications as far as public health response (e.g. contact tracing, isolation, chemoprophylaxis or environmental investigation).

To better improve on existing surveillance, the overall goals of the UNEX project are to:

- Provide a simplified, uniform algorithm for local health departments and coroners/medical examiners to identify and workup fatal cases of possible infectious etiology
- Enhance the quality of clinical specimens available for testing;
- Obtain accurate and standardized data and reporting for potential infectious disease deaths in a timely manner
- Systematically evaluate role and utility of new techniques for identifying infectious pathogens.
- Improve collaboration between clinicians, pathologists and public health practitioners;
- Build an infrastructure to evaluate cases of unexplained infectious disease, respond to clusters of unexplained illness, and detect emerging pathogens or possible acts of biologic terrorism

To support the identification of organism-specific etiologies in infectious disease-related deaths, the UNEX Project, in conjunction with the CDC and the CDPH MDL, can provide assistance with the diagnostic workup. As the state reference laboratories, the VRDL and MDL offer a broad range of diagnostic testing for viral, bacterial and rickettsial pathogens, including molecular-based assays such as PCR and sequencing, culture, serologic testing and electron microscopy. The CDC Infectious Disease Pathology Unit offers PCR, immunohistochemical staining and expert analysis by infectious disease pathologists.

Case Definition:
The UNEX surveilance project uses the following criteria:

- Fatal
- Hallmarks of infection (e.g. fever, leukocytosis)
- History suggestive of community-acquired infection
- An unknown etiology after initial in-hospital testing

In the UNEX project, participating local health departments will be encouraged to work with the medical examiners, coroners and pathologists in their jurisdiction to systematically assess and report any fatal cases of possible infectious etiology. UNEX will encourage the use of the New Mexico Office of the Medical Investigator (NM OMI) pathology-based syndromic surveillance system called Med-X. Med-X uses a set of antemortem signs/symptoms identified through death scene investigation, contact interviews or medical record review (e.g., fever, acute encephalopathy, acute diarrhea, new rash, acute flaccid paralysis, new onset jaundice) to identify cases that warrant an autopsy for infectious etiologies. Based on gross and histologic findings at autopsy, the case is classified into a set of pathologic syndromes (e.g., community-acquired pneumonia, myocarditis, encephalitis, sepsis syndrome). Gross findings, in combination with antemortem signs/symptoms, guide specimen collection and laboratory evaluation. These symptoms and syndromes provide an initial basis for defining a differential diagnosis, directing microbiologic testing and reporting the case to UNEX. Local health departments are supplied with Autopsy Specimen Collection Kits for their MEs and coroners to encourage collection of optimal pathology specimens.

The participating medical examiners, pathologists and clinicians are encouraged to take advantage of the state-of-the art testing available at both CDPH and CDC. As the state reference laboratories, the VRDL and MDL offer a broad range of diagnostic testing for viral, bacterial and rickettsial pathogens, including molecular-based assays such as PCR and sequencing, culture, serologic testing and electron microscopy. Examples of tests available include PCR and/or serologic testing for many viral and bacterial pathogens including influenza, RSV, parainfluenza viruses, *M. pneumoniae*, *Chlamydiae pneumoniae*, legionellosis, enterovirus, herpes and varicella viruses, and *Neiserria meningitis*, as well as more unusual and recently discovered agents such as human metapneumovirus, bocavirus, hantavirus, Q fever, pox viruses, RMSF and erhlichiosis, to name a few. In the appropriate clinical setting with sterile site specimens, broad range testing for all bacterial families with 16S PCR can be performed. In addition, electron microscopy studies of appropriate skin biopsy and stool specimens can assist with identification of infectious etiologies. Pathology tissues are forwarded to the CDC IDPA for testing, including immunohistochemical analysis. For best results, early consultation is encouraged in order to obtain the optimal clinical specimens that will provide the best yield with diagnostic testing. A specimen bank is maintained for all cases in the event that future diagnostic tests became available.

In addition to participating counties, UNEX will also assist any county who requests help with cases of unexplained deaths due to infectious etiology. By offering this service, the UNEX project hopes to serve as a source of information for local health care and public health professionals statewide, by providing clinical advice and technical assistance with evaluation of the differential diagnosis for unexplained deaths that may be due to an infectious etiology.

In 2007, 18 cases of unexplained death due to possible infectious etiology were referred to VRDL and MDL for further testing from 9 counties. The age range was 1 month to 64 years with median age of 1 year. Cases were classified by clinical syndrome, including Sudden Infant Death Syndrome (7), lower respiratory tract infection/acute respiratory distress syndrome (4), myocarditis (4), febrile rash illness (1) and other (3). Eleven cases (61%) had an etiology identified, including seven with lower respiratory tract infection [Streptococcus pneumoniae (2), and one each of hantavirus, anaplasma and

rhinovirus], one with myocarditis (enterovirus) and four with febrile rash illness [dengue (2), *Mycoplasma* (1) and gram positive rod sepsis (1)].

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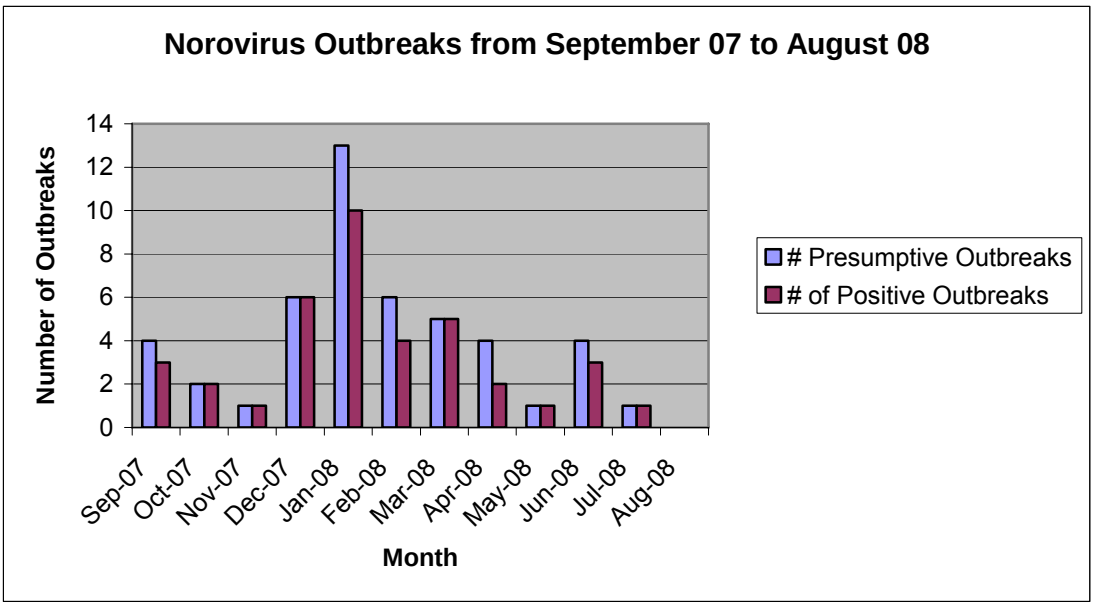
Viral Gastrointesteritis (Norovirus) Outbreaks: 2007-08

Background:
In collaboration with California local public health laboratories (LPHL), the VRDL conducts statewide laboratory surveillance for norovirus outbreaks, including performing molecular characterization to help trace the source and spread of outbreaks and to analyze currently circulating and emerging strains. LPHLs perform initial testing of outbreaks suspected to be due to norovirus. Currently, 24 LPHLs offer testing, including Contra Costa, El Dorado, Fresno, Los Angeles, Long Beach, Marin, Monterey, Orange, Placer, Riverside, Sacramento, San Bernardino, San Diego, San Francisco, San Joaquin, San Luis Obispo, San Mateo, Santa Barbara, Santa Clara, Shasta, Solano, Sonoma, Tulare and Ventura. A portion of positive samples are then forwarded to the VRDL for molecular analysis. The VRDL will only accept specimens for norovirus testing in outbreak settings and only following request from the local health department; testing of individual cases not associated with an outbreak is accepted only following consultation with the VRDL.

Recent activity:
The 2007-08 season was a relatively mild norovirus season. From September 2007 through August 2008, 239 clinical specimens from 47 non-foodborne outbreaks of acute gastroenteritis had been tested. Of these, 148 clinical samples (62%) from 38 outbreaks (81%) tested positive for norovirus. In addition, 55 RNA extracts were further characterized from 31 outbreaks tested at LPHLs. Approximately 90% of viruses were characterized as genogroup GII. Over 90% of samples came from long-term care facilities (LTCF); a small percentage of samples also came from restaurants, prisons and schools/school camps. A summary graph of outbreaks tested at VRDL is shown below:

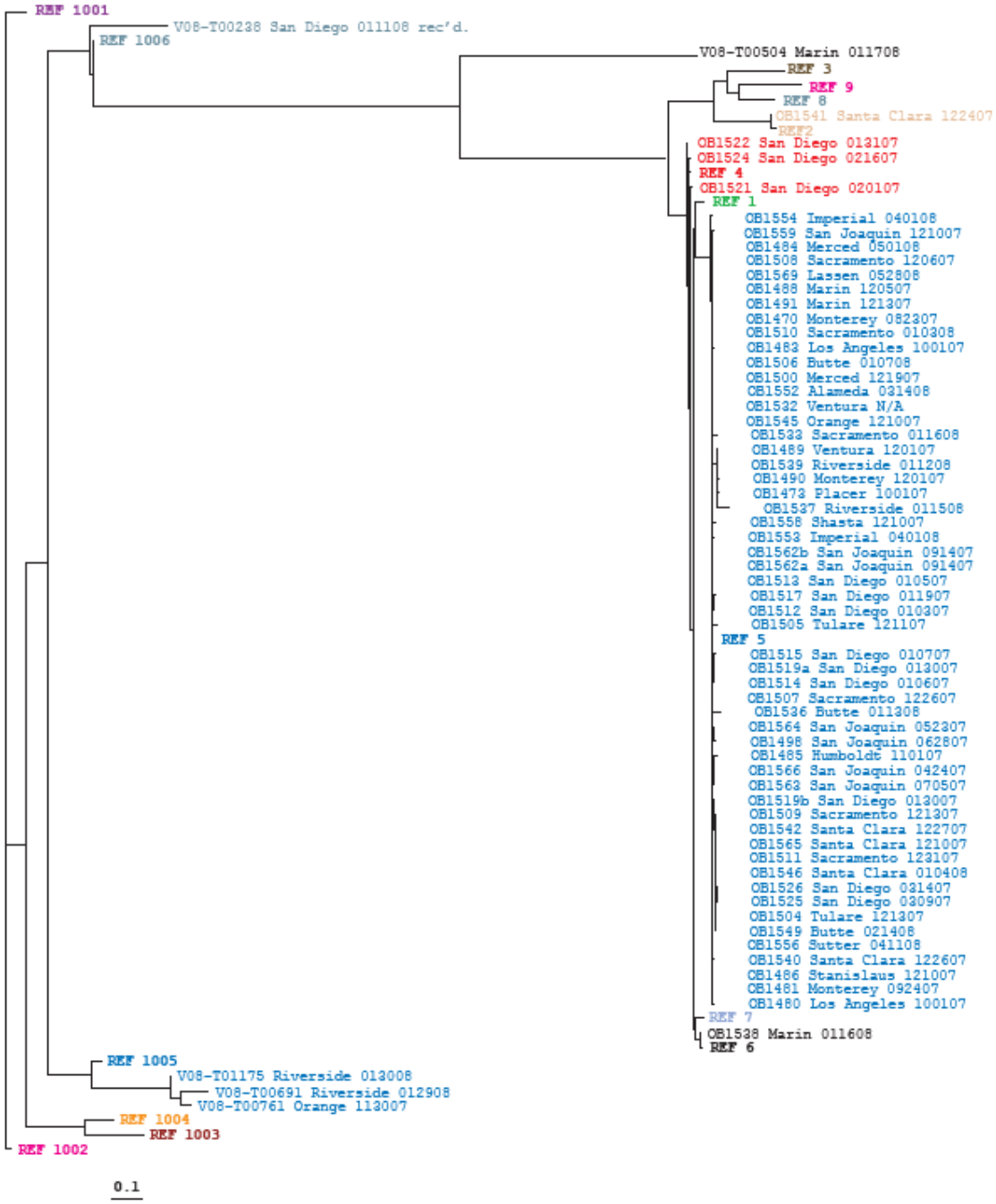


Molecular strain characterization:
Due primarily to the inability to grow norovirus in-vitro, no simple serological or biological assay is available. Norovirus classification is currently based on phylogenetic grouping based on viral sequences. Molecular analysis of norovirus nucleic acid sequences has shown they exist in great diversity with at least five major phylogenetic genogroups, including genogroups GI, G2 and G4 which are known to infect humans. Within the genogroups are clusters (or genotypes).

From year to year, the VRDL monitors the distribution patterns for noroviruses which fall into the specific clusters and analyzes how these clusters change. The VRDL has characterized several hundred outbreaks statewide; phylogenetic analysis of isolates collected since 2005 using the real-time PCR to identify genogroup and a region D-specific PCR to identify finer distinctions has consistently showed the distribution of the isolates into the two clades representing genogroups I and II. The predominant strain identified has been Genogroup 2, cluster 4; a similar pattern of G2, cluster 4 is predominant throughout the US and worldwide.

During the 2007-2008 season, there was a single predominant strain of norovirus genogroup II, designated CA2, detected in California. Strain CA2 is a GII.4 genotype norovirus closely related to GII.4/Nijmegen115/206/NL (REF 5 in the phylogenetic tree, also known as Minerva as reported by the CDC and GII.4-2006b strain as reported in Europe). In the 2005-2006 season in California, there was a predominance of strain CA1, another GII.4 genotype of norovirus closely related to GII.4/Terneuzen70/2006/NL (REF 4 in the phylogenetic tree, also known as Laurens strain as reported by the CDC and GII.4-2006a as reported in Europe). However, during the 2006-2007, CA2 emerged and in the 2007-2008 season continued to be the predominant variant of norovirus circulating in California. This trend follows the global distribution pattern of norovirus. In January 2008, the Foodborne Viruses in Europe network also reported that the norovirus GII.4 2006b (in Japan the 034-like and in UK V6) variant was dominant in early seasonal surveillance.

Norovirus 2007-2008 season



Disclaimer: *The data collected through the VRDL on viral gastrointestinal outbreaks are based upon voluntary participation and submission of samples from the local and county public health laboratories and, therefore, represent incomplete data for California.*

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West Nile Virus (WNV) Surveillance

WNV is a mosquito-borne arbovirus that is commonly found in Africa, West Asia, and the Middle East. WNV was first detected in the United States in New York in 1999, and has since spread to 48 states including California, where activity was first detected in 2003. The virus is typically transmitted through the bite of an infected mosquito. Mosquitoes become infected when they feed on infected birds.

Most persons infected with WNV do not develop clinical illness or symptoms. However, approximately 20% of people develop symptoms compatible with what is known as West Nile fever. About one in 150 people infected with WNV develop severe neuroinvasive illness. Although milder WNV illness often improves without treatment, those cases with severe WNV illness may require hospitalization and supportive care. However, there is no specific treatment for WNV.

In 2002, a regional public health laboratory network was established in California to enhance human WNV testing and surveillance efforts throughout the state. The regional public health network consists of the VRDL as well as over 30 LPHLs that have adopted WNV testing capability. VRDL also collaborates with reference laboratories such as the regional laboratories of Kaiser Permanente to ascertain additional suspect WNV cases. LPHLs and other reference laboratories often forward specimens to VRDL for repeat or additional testing.

Serum and cerebrospinal fluid specimens are requested for individuals with: (a) encephalitis; (b) aseptic meningitis; (c) acute flaccid paralysis, atypical Guillain-Barré syndrome, or transverse myelitis; or (d) febrile illness compatible with the West Nile fever syndrome and lasting $\geq$ 7 days. The enzyme immunoassay (EIA) is the initial test performed on all specimens at VRDL. Other WNV testing performed at VRDL includes the immunofluorescence assay (IFA), WNV PCR, and confirmatory plaque reduction neutralization testing (PRNT).

The first cases of WNV in California were reported in 2003, in three residents of southern California. The number of cases subsequently rose dramatically, with 779 cases reported in 2004 (incidence 2.30 cases per 100,000) and 880 cases reported in 2005 (incidence 2.60 cases per 100,000). California reported the greatest number of cases nationwide in both those years. By the end of 2007, a total of 2,317 WNV cases had been reported in California since its introduction to the state.

In 2006, VRDL tested over 1,500 individuals for WNV. More than 3,000 different assays were performed for these suspect cases (Table 1). In 2007, over 2,600 assays were performed for 1,333 individuals. Positive WNV lab results are reported back to the submitter as well as to the local health department in the individual's county of residence. Since WNV infection is a reportable disease, local health departments must investigate laboratory or provider reports of WNV infection. Since WNV antibodies can cross-react with other flavivirus antibodies and WNV IgM antibodies can persist for up to one year, local health departments must interpret positive laboratory reports with caution.

Table 1. West Nile Virus testing at VRDL, 2004-2007.

	2004	**2005**	**2006**	**2007**
Number of individuals tested at VRDL	2398	2210	1580	1333
Number of specimens tested	2722	2485	1658	1436
Number of assays performed	6491	4771	3051	2682
West Nile Virus EIA IgM	2740	2503	1657	1435
West Nile Virus EIA IgG	2209	1621	1003	897
West Nile Virus IFA IgM	306	218	112	111
West Nile Virus IFA IgG	306	218	112	111
West Nile Virus PCR	15	9	10	11
West Nile Virus PRNT	483	86	64	45
St. Louis Encephalitis PRNT	431	83	62	44
Dengue PRNT	1	32	31	17
Western Equine Encephalitis PRNT	0	1	0	11

In California, WNV cases have typically been reported from June through December, with the greatest number of cases reported in August and September. Of the 2,317 WNV cases reported from 2003 to 2007, 1,355 (58%) were male, and the age of cases has ranged from 2 to 96 years (median=52 years), with the incidence of WNV neuroinvasive disease and WNV-associated fatalities highest in older individuals. A total of 76 WNV-associated fatalities were reported from 2004-2007.

Among the cases reported in California, risk factors associated with developing severe West Nile neuroinvasive disease as compared to West Nile fever include older age, male gender and diabetes mellitus. Other mosquito-borne arboviruses that are established in the United States typically have continued background transmission with periodic epidemics; a similar trend is expected with WNV in coming years.

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VRDL Publications and Abstracts, 2007-2008

Viral Immunoserology Section

Abstracts/Presentations

1. C Preas, JK Louie, MD, S Honarmand, MS, B Forghani, PhD, CA Glaser, DVM, MD. Herpes Simplex Encephalitis (HSE): Diagnostic Experience of the California Encephalitis Project. Twenty-Third Annual Clinical Virology Symposium, Clearwater Beach, FL, April 29-May 2, 2007.
2. Natasha Huntziker, BA, Laura Christie, MD, Mi-Khin K Soe, MPH, Somayeh Honarmand, MS, Carol Glaser, MD, DVM. Epstein Barr Virus Testing in Encephalitis Patients: Comparison of Real Time Polymerase Chain Reaction and Serologic Methods. Twenty-Third Annual Clinical Virology Symposium, Clearwater Beach, FL, April 29-May 2, 2007
3. Z Amad, C Cossen, G Cosentino, C Jean, C Preas, M Shahkarami, B Forghani. Use of Immunoglobulin G Avidity to Characterize Diagnostic Samples for Early and Late Antibody to West Nile Virus. Twenty-Third Annual Clinical Virology Symposium, Clearwater Beach, FL, April 29-May 2, 2007
4. Eleanore D. Sternberg, Sharon L. Messenger, David Schnurr. Survey of Bat-Associated Rabies Virus in California. Twenty-Third Annual Clinical Virology Symposium, Clearwater Beach, FL, April 29-May 2, 2007.

Viral Isolation Section

Peer-reviewed publications

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5. Kiang D, Yagi S, Kantardjieff KA, Kim EJ, Louie JK, Schnurr DP. Molecular characterization of a variant rhinovirus from an outbreak associated with uncommonly high mortality. J Clin Virol. 2007 Mar;38(3):227-37. Epub 2007 Feb 2.
6. Kistler A, Avila PC, Rouskin S, Wang D, Ward T, Yagi S, Schnurr D, Ganem D, Derisi JL, Boushey HA. Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. J Infect Dis. 2007 Sep 15;196(6):817-25. Epub 2007 Aug 6.
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8. Louie JK, Schnurr DP, Pan CY, Kiang D, Carter C, Tougaw S, Ventura J, Norman A, Belmusto V, Rosenberg J, Trochet G. A summer outbreak of human metapneumovirus infectionin a long-term-care facility. J Infect Dis. 2007 Sep 1;196(5):705-8. Epub 2007 Jul 13.
9. Oberste MS, Maher K, Nix WA, Michele SM, Uddin M, Schnurr D, Al-Busaidy S, Akoua-Koffi C, Pallansch MA. Molecular identification of 13 new enterovirus types, EV79-88, EV97, and

EV100-101, members of the species Human Enterovirus B. Virus Res. 2007 Sep;128(1-2):34-42. Epub 2007 May 7.
10. Louie JK, Schnurr DP, Guevara HF, Honarmand S, Cheung M, Cottam D, Yeh E, Wold L, Boston EJ, Tang J, Cummings KC, Donovan RM, Schechter R, Rosenberg J, Walter LJ, Chapman JA, Brenner PR, Baxter RP, Glaser CA. Creating a model program for influenza surveillance in California: results from the 2005-2006 influenza season. Am J Prev Med. 2007 Oct;33(4):353-7.

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1. Vu M, Yeh E, Schnurr D. Comparison of sensitivity and specificity of a new enterovirus group reactive monoclonal antibody reagent from Diagnostic Hybrids to reagents from Chemicon and Dako. 23rd Annual Clinical Virology Symposium, Clearwater Beach, FL. 2007.
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Retrovirus Diagnostic Section

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4. Hanson CV, Wang C-Y, Finstad CL, Chang T-Y, Hung C-H, Fang X-D, Xia W-G, Salas M. Therapeutic HIV-RC Vaccine Targeting Host Cell CD4. AIDS Vaccine 07. Seattle, Washington, August 2007.

Epidemiology Support/HIV Section

Peer-reviewed publications

1. Planque S, Mitsuda Y, Taguchi H, Salas M, Morris MK, Nishiyama Y, Kyle R, Okhuysen P, Escobar M, Hunter R, Sheppard HW, Hanson C, Paul S. Characterization of gp120 hydrolysis by IgA antibodies from humans without HIV infection. AIDS Res Hum Retroviruses. 2007 Dec;23(12):1541-54.
2. Rademeyer C, Moore PL, Taylor N, Martin DP, Choge IA, Gray ES, Sheppard HW, Gray C, Morris L, Williamson C; HIVNET 028 study team. Genetic characteristics of HIV-1 subtype C envelopes inducing cross-neutralizing antibodies. Virology. 2007 Nov 10;368(1):172-81.
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Epidemiology and Medical Liaison Section

Peer-reviewed publications

1. Ramirez-Avila L, Slome S, Schuster FL, Gavali S, Schantz PM, Sejvar J, Glaser CA. Eosinophilic meningitis due to Angiostrongylus and Gnathostoma species. Clin Infect Dis 2009; 48(3):322-7.
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9. Bloch KC, Glaser C. Diagnostic approaches for patients with suspected encephalitis. Curr Infect Dis Rep. 2007 Jul;9(4):315-22.
10. Janner DL, Probert WS, McClure CD. Ribosomal DNA Assay of Culture-Negative Streptococcus pneumoniae Meningitis. Pediatr Neurol. 2007 Jul;37(1):55-8.
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12. Louie JK, Schnurr DP, Guevara HF, Honarmand, S, Cheung, M, Cottam D, Yeh E, Wold W, Boston EJ, Tang J, Cummings KC, Donovan RM, Schechter R, Rosenberg J, Walter LJ, Chapman JA, Brenner PR, Baxter R, Glaser CA. Creating a Model Program for Influenza

Surveillance in California: Results from the 2005-06 Influenza Season. Am J Prev Med. 2007 Oct;33 (4):353-7
13. Louie JK, Schnurr DP, Pan C, Kiang D, Carter C, Tougaw S, Ventura J, Norman A, Belmusto V, Rosenberg J, Trochet G. A Summer Outbreak of Human Metapneumovirus Infection in a Long-Term Care Facility. J Infect Dis. 2007 Sep 1;196(5):705-8.
14. Jean CM, Honarmand S, Louie JK, Glaser CA. Risk factors for West Nile virus neuroinvasive disease: The California experience, 2005. Emerg Infect Dis. 2007 Dec;13(12):1918-20
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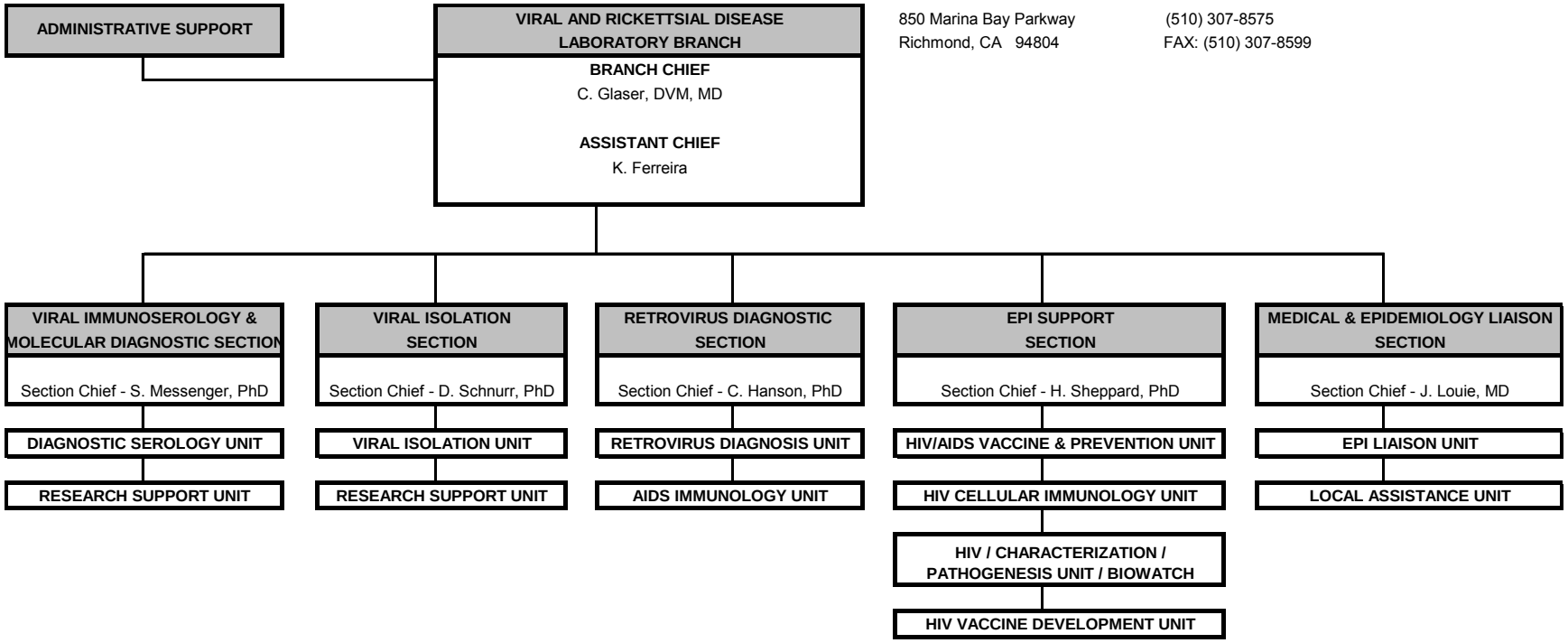
3. Preas C, Louie JK, Honarmand S, Forghani B, Glaser CA. Herpes Simplex Encephalitis (HSE): Diagnostic experience of the California Encephalitis Project. 23rd Clinical Virology Symposium. Clearwater Beach, Florida. April 29-May 2, 2007.
4. Huntziker N, Christie LJ, Soe M-KK, Honarmand S, Glaser CA. Epstein Barr Virus Testing In Encephalitis Patients: Comparison of Real Time Polymerase Chain Reaction and Serologic Methods. 23rd Clinical Virology Symposium. Clearwater Beach, Florida. April 29-May 2, 2007.

VRDL Organizational Chart