



## Laboratory Testing for Spotted Fever Rickettsiosis

A newly described eschar-associated illness has been identified in California caused by Spotted Fever Group (SFG) *Rickettsia* 364D and transmitted with tick bite. The most prominent clinical feature of *Rickettsia* 364D infection is the development of an isolated ulcer with raised erythematous margins and core black eschar, usually with surrounding generalized edema and erythema, which develops 3-14 days at the site of a known or presumed tick bite. Rickettsial illness caused by *Rickettsia* 364D is reportable under California Code of Regulations Title 17.

The California Department of Public Health (CDPH) Viral and Rickettsial Disease Laboratory (VRDL) encourages submission of specimens from suspected SFG *Rickettsia* cases who meet the CDC clinical case definition.

**CDC spotted fever rickettsiosis clinical case definition:** An illness characterized by any reported fever and one or more of the following:

- Rash
  - Eschar
  - Headache
  - Myalgia
  - Anemia
  - Thrombocytopenia, or
  - Any hepatic transaminase elevation
- To confirm a case of SFG *Rickettsia*, the VRDL uses the reference standard IFA test on paired sera.
  - To specifically identify *Rickettsia* 364D for surveillance purposes, direct detection and sequencing of *Rickettsia* DNA is necessary.

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

### Specimen collection:

**Minimum specimen requirement:** Paired serum specimens. Sera (5-10 cc) should be collected in a red top or tiger top tube.

- Acute serum should be taken in the 1st week of illness (or within ~7-10 days post onset)
- Convalescent serum should be taken at least 3-4 weeks following the acute sample

### **Additional desired specimens, as available:**

To assist with surveillance studies of *Rickettsia* 364D, for ALL cases where rickettsial infection with associated eschar is suspected, the following samples should also be submitted (as available):

- **Eschar/Scab** (if present)
- **Swab(s)** of open lesions, pustules or vesicles
- **Ticks** associated with suspected cases may also be submitted for identification and PCR testing.

General Eschar/Swab Sample Protocol (Ideally *prior* to antibiotic treatment):

- Clean eschar with disinfectant. Wipe area with sterile gauze and sterile saline.
- With sterile tweezers, lift scab area partially or completely. If scab is removed, place in **dry** sterile vial. Label with patient's name and date.
- Alternatively, using a dry sterile Dacron swab, obtain a sample of the ulcerated area under scab by swabbing with gentle rotation and pressure. Two swabs are requested. Place Dacron swab(s) in sterile **dry** container with lid. Label with patient's name and date. Cover sampled area with sterile covering.

### **Specimen storage (all specimen types):**

Transport samples with cold packs as soon as possible following collection. If samples cannot be transported immediately, they can be held at 4°C for up to 72 hours before shipping. Otherwise, specimens should be frozen, preferably at -70°C, and shipped on dry ice. Avoid repeat freeze-thaw cycles.

### **Shipping:**

***You must notify your Local Health Department (LHD)*** that you wish to submit samples. Many LHDs will want samples sent to them to forward to VRDL; others will authorize shipping directly to VRDL. Samples sent without LHD authorization will not be tested.

***Specimen submission form is required.*** A general purpose specimen submittal form can be found at:

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

#### **Ship human clinical samples to:**

Specimen Receiving  
Viral and Rickettsial Disease Laboratory  
California Department of Public Health  
850 Marina Bay Pkwy  
Richmond, CA 94804

#### **Ship ticks to:**

Specimen Receiving  
c/o VBDS  
California Department of Public Health  
850 Marina Bay Pkwy  
Richmond, CA 94804

### **Laboratory testing and interpretation:**

**Serology:** The indirect immunofluorescence assay (IFA).

- A four-fold rise in IgG antibody titer reactive for *R. rickettsii* or other rickettsial antigen will confirm acute infection.
- In the majority of patients, detectable antibody titers (IgM or IgG) are usually observed 7-10 days after illness onset, however;

- Antibody titers may not be detected in the first week of illness. A negative test result during this time does not rule out SFG *Rickettsia* infection.
- Patients infected with certain imported rickettsiae might not demonstrate increased titers until 4 weeks after illness onset.
- Elevations in rickettsial IgM antibody titers are less specific; therefore, samples positive for IgM should be screened for IgG antibody to confirm specificity.
- For rickettsial agents, antibody titers (including IgM) may persist in some individuals for months to years after the original exposure. Positive antibody titers in the absence of clinical history compatible with SFG *Rickettsia* may indicate past exposure.

### **Molecular Testing:** PCR and sequencing

Currently, VRDL is validating molecular testing to confirm IFA results as well as to specifically identify newly described rickettsial agents that may be present in California. These new agents include *Rickettsia* 364D (i.e., recently recognized as a pathogen in humans). VRDL uses a PCR test followed by sequencing of the PCR product to confirm the specific identity of a SFG *Rickettsia*.