Supplemental Table 1. Exclusivity panel of nucleic acids

| Organism | Strain | Source |
|---|---|---|
| *Rickettsia* species | 364D | CDC |
| *Rickettsia akari* | Kaplan | CDC |
| *R. amblyommatis* | GAT-30V | CDC |
| *R. asembonensis* | NMRC11 | CDC |
| *R. massiliae* | AZT80 | CDC |
| *R. parkeri* | Maculatum 20 | CDC |
| *R. prowazekii* | Madrid II | CDC |
| *R. rhipicephali* | 20-4164 | CDC |
| *R. rickettsii* | Sheila Smith | CDC |
| *R. sibirica* | 246 | BEI Resources |
| *R. tillamookensis* | Tillamook 23 | CDC |
| *Anaplasma phagocytophilum* | NCH-1 | BEI Resources |
| *Erhlichia chaffeensis* | St. Vincent | BEI Resources |
| *Orientia tsutsugamushi* | Karp | CDC |
| Coxsackievirus A6 | 05871 | CDPH |
| Coxsackievirus A16 | 02550 | CDPH |
| Enterovirus A71 | 03669 | CDPH |
| Dengue virus type 1 | HAW | CDPH |
| Dengue virus type 2 | NGC | CDPH |
| Dengue virus type 3 | H87 | CDPH |
| Dengue virus type 4 | H41 | CDPH |

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|---|---|---|
| Herpes herpesvirus 1 | 019354 | CDPH |
| Herpes herpesvirus 2 | 020806 | CDPH |
| Human herpesvirus 3 | Batson | CDPH |
| Human herpesvirus 4 | B95-8 | CDPH |
| Human herpesvirus 5 | TC-844 | CDPH |
| Human herpesvirus 6 | Z-29 | CDPH |
| Human immunodeficiency virus type 1 | 23135 | CDPH |
| Measles virus | 04850 | CDPH |
| Rubella virus | 00182 | CDPH |
| West Nile virus | TC 86300 | CDPH |
| Zika virus | PRVABC59 | CDPH |
| *Bartonella bacilliformis* | ATCC 35685 | CDPH |
| *Bartonella henselae* | ATCC 49882 | CDPH |
| *Bartonella quintana* | ATCC 51694 | CDPH |
| *Coxiella burnetii* | Nine mile | CDPH |
| *Neisseria meningitidis* | M11095 | CDPH |
| *Neisseria gonorrhea* | WHO_Z | CDPH |
| *Treponema pallidum* | A3-2 | CDPH |
| *Streptococcus pyogenes* | ATCC 21059 | CDPH |
| *Salmonella typhi* | 80-2002 | CDPH |
| *Staphylococcous aureus* | AR_0225 | CDPH |

CDC = Centers for Disease Control and Prevention; CDPH = California Department of Public Health

Supplemental Table 2. *Rickettsia* species and subspecies sequences used for 23S rRNA analyses

| Species/Subspecies | Strain | GenBank Accession Number |
|---|---|---|
| *R. aeschlimannii* | MC16 | CCER01000011.1 |
| *R. africae* | ESF-5 | CP001612.1 |
| *R. akari* | Hartford | CP000847.1 |
| *R. amblyommatis* | GAT-30V | CP003334.1 |
| *R. asembonensis* | Perak | CP116496.1 |
| *R. asiatica* | Maytaro1284 | AP019563.1 |
| *R. australis* | Cutlack | CP003338.1 |
| *R. bellii* | RML369-C | CP000087.1 |
| *R. canadensis* | CA410 | CP003304.1 |
| *R. conorii subsp. conorii* | Malish 7 | AE006914.1 |
| *R. conorii subsp. indica* | ITTR | AJHC01000003.1 |
| *R. conorii subsp. israelensis* | ISTT CDC1 | AJVP01000009.1 |
| *R. conorii subsp. raoultii* | Khabarovsk | CP010969.1 |
| *R. felis* | URRWXCal2 | NR_076359.1 |
| *R. fournieri* | AUS118 | OFAL01000006.1 |
| *R. gravesii* | BWI1 | AWXL01000005.1 |
| *R. heilongjiangensis* | 054 | CP002912.1 |
| *R. helvetica* | C9P9 | AICO01000001.1 |
| *R. honei* | RB | AJTT01000002.1 |
| *R. hoogstraalii* | Croatica | CCXM01000001.1 |
| *R. japonica* | YH | AP011533.1 |

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|---|---|---|
| *R. massiliae* | MTU5 | CP000683.1 |
| *R. monacensis* | IrR/Munich | LN794217.1 |
| *R. montanensis* | OSU 85-930 | CP003340.1 |
| *R. parkeri* | Portsmouth | CP003341.1 |
| *R. peacockii* | Rustic | CP001227.1 |
| *R. prowazekii* | Breinl | CP004889.1 |
| *R. rhipicephali* | 3-7-female6-CWPP | CP003342.1 |
| *R. rickettsii* | Iowa | CP000766.3 |
| *R. sibirica* | 246 | AABW01000001.1 |
| *R. slovaca* | D-CWPP | CP003375.1 |
| *R. tamurae subsp. buchneri* | PalLab | CP113531.1 |
| *R. tamurae subsp. tamurae* | AT-1 | CCMG01000015.1 |
| *R. tillamookensis* | Tillamook 23 | CP060138.2 |
| *R. typhi* | Wilmington | NR_076209.1 |

Nested 23S rRNA RT-PCR Sequencing Reference Test

A nested 23S rRNA RT-PCR sequencing assay was developed and used to resolve discrepant results between the 23S rRNA RT-rtPCR assay and the *ompB* rtPCR assay. The outer RT-PCR was performed using the PrimeScript One-Step PCR kit (Takara Bio) in a 50 µL reaction volume consisting of 10 µL of nucleic acids and primers Rk_23S_1137F (5'-GGCTCAAGTCATGTACCGAA-3') and Rk_23S_1609R (5'-ACAGGTCATCTTTCTTCCGA-3') each at a concentration of 600 nM. RT-PCR was performed using a T-100 thermal cycler (Bio-Rad Laboratories, Hercules, CA) with cycling parameters consisting of 50°C for 30 min, 94°C for 2 min, 50 cycles at 94°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min, and a final hold at 72°C for 2 min. The inner PCR was conducted in a reaction volume of 25 µL using 1X Q5 Hot Start, High Fidelity master mix (New England Biolabs, Ipswich, MA), primers Rk_23S_1143 (5'- AGTCATGTACCGAAGATGCG-3') and Rk_23S_1606R (5'- GGTCATCTTTCTTCCGAAGTTAC-3) each at a concentration of 300 nM, and 1 µL of the outer RT-PCR product. Amplification was performed in a T-100 thermal cycler using cycling parameters consisting of 98°C for 30 sec, 35 cycles at 98°C for 5 sec, 61°C for 20 sec, and 72°C for 30 sec, and a hold at 72°C for 2 min. An expected amplified product size of 464 bp was assessed using the D1000 ScreenTape system and TapeStation 4200 instrument (Agilent Technologies, Santa Clara, CA). Bidirectional Sanger sequencing using primers Rk_23S_1143F and Rk_23S_1606R was performed by a commercial laboratory (Sequetech Corporation, Mountain View, CA). Sequences were edited and assembled with Geneious v10 software (Biomatters Inc., Boston, MA) and a BLAST search of the NCBI nr/nt database performed for *Rickettsia* species identification.