Article DOI: http://dx.doi.org/10.3201/eid2112.150981

# Life-Threatening Sochi Virus Infections, Russia

## Technical Appendix

**Technical Appendix Table 1.** Details of serodiagnostic data (reciprocal antibody titers) of the 62 patients*

| Patient no. | No. serum samples | Sampling day after disease onset | IFA | | | | ELISA | | | | FRNT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | PUUV | | DOBV | | | DOBV | | | | |
| | | | PUUV | DOBV | HTNV | SEOV | IgM | IgG | IgM | IgG | PUUV | Sochi | Kurkino | Dobrava | HTNV | SEOV |
| 1 | 1,293 | 27 | <16 | 1024 | 1024 | 512 | <128 | <128 | 16384 | 32768 | <40 | 1280 | 640 | 640 | | |
| | 1312 | 104 | <16 | 2048 | 512 | 512 | <128 | <128 | 2048 | 8192 | <40 | 1280 | 160 | 160 | 160 | <80 |
| 2 | 1308 | 22 | <16 | 2048 | 1024 | 1024 | 256 | <128 | 4096 | 4096 | <40 | 640 | 80 | | 160 | 80 |
| | 1310 | 50 | <16 | 8192 | 4096 | 4096 | | | | | <40 | 1280 | 640 | 640 | 160 | 160 |
| | 4708† | 5 y 9 m | 64 | 2048 | 2048 | 2048 | | | | | <40 | 2560 | 1280 | 2560 | 80 | 40 |
| 3 | 1307 | 15 | <16 | 4096 | 2048 | 2048 | <128 | <128 | 8196 | 8196 | <40 | 640 | 320 | 320 | 160 | 160 |
| 4 | 1335 | 17 | 64 | 8192 | 4096 | 4096 | 512 | 512 | 8192 | 16384 | <40 | 1280 | <80 | 80 | | |
| | 1336 | 82 | <32 | 4096 | 2048 | 1024 | 1024 | 512 | 4096 | 32768 | <40 | 640 | 320 | 640 | <80 | 160 |
| | 4,709† | 5 y 9 m | 64 | 4096 | 4096 | 2048 | | | | | <40 | 5120 | 1280 | 2560 | 160 | 80 |
| 5 | 1339 | 11 | <32 | 4096 | 2048 | 2048 | 256 | <128 | 8192 | 8192 | <40 | 320 | 80 | | | |
| 6 | 1642 | 3 | <16 | 2048 | 1024 | 512 | <128 | <128 | 8196 | 2048 | | | | | | |
| | 4716† | 5 y 3 m | 128 | 4096 | 4096 | 4096 | | | | | <40 | 5120 | 1280 | 2560 | 160 | 160 |
| 7 | 1686 | 16 | <16 | 2048 | 1024 | 512 | <128 | <128 | 2048 | 2048 | <40 | 640 | 80 | 80 | | |
| 8 | 4712 | 9 | <16 | 512 | 256 | 256 | <128 | <128 | 2048 | 512 | | | | | | |
| 9 | 3507 | 15 | 64 | 4096 | 2048 | 2048 | <128 | <128 | 8196 | 8196 | <40 | 640 | 160 | 320 | | |
| | 4713† | 1 y 5 m | 1024 | 8192 | 4095 | 4096 | | | | | <40 | 20480 | 5120 | 10240 | 320 | 160 |
| 10 | 3830 | 13 | 128 | 8192 | 2048 | 2048 | 512 | 256 | 16384 | 8192 | <40 | 640 | 160 | 640 | | |
| | 4714† | 1y 5m | 512 | 16384 | 16384 | 8192 | | | | | <40 | 20480 | 5120 | 5120 | 80 | 40 |
| 11 | 3692 | 30 | 64 | 4096 | 4096 | 2048 | 256 | 256 | 4096 | 16384 | <40 | 1280 | 160 | <160 | | |
| 12 | 3693 | 10 | 32 | 2048 | 1024 | 1024 | <128 | <128 | 16384 | 8192 | | | | | | |
| | 3694 | 18 | 64 | 2048 | 2048 | 2048 | | | | | <40 | 640 | <80 | 80 | | |
| 13 | 3496 | 9 | <16 | 4096 | 4096 | 2048 | <128 | <128 | 16384 | 16384 | | | | | | |
| 14 | 3802 | 11 | 64 | 4096 | 2048 | 2048 | <128 | <128 | 8192 | 2048 | <40 | 1280 | 320 | 640 | | |
| | 4711† | 1 y 6 m | 512 | 8192 | 8192 | 4096 | | | | | <40 | 20480 | 20480 | 20480 | 160 | 80 |
| 15 | 3778 | 10 | 64 | 8192 | 4096 | 4096 | <128 | <128 | 65536 | 8192 | | | | | | |
| 16 | 3824 | 11 | 64 | 8192 | 8192 | 4096 | <128 | <128 | 16384 | 16384 | | | | | | |
| 17 | 3813 | 4 | 64 | 8192 | 2048 | 2048 | <128 | <128 | 16384 | 16384 | | | | | | |
| | 4710† | 1 y 5 m | 64 | 2048 | 2048 | 2048 | | | | | <40 | 10240 | 5120 | 5120 | | |
| 18 | 3861 | 16 | 256 | 8192 | 4096 | 4096 | <128 | <128 | 8192 | 2048 | <40 | 640 | 640 | 640 | | |

| Patient no. | No. serum samples | Sampling day after disease onset | IFA | | | | ELISA | | | | FRNT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | PUUV | | DOBV | | | DOBV | | | | |
| | | | PUUV | DOBV | HTNV | SEOV | IgM | IgG | IgM | IgG | PUUV | Sochi | Kurkino | Dobrava | HTNV | SEOV |
| | 4715† | 1 y 4 m | 128 | 8192 | 8192 | 4096 | | | | | <40 | 5120 | 2560 | 5120 | 80 | 40 |
| 19 | 3855 | 9 | <16 | 4096 | 2048 | 2048 | <128 | <128 | 16384 | 2048 | | | | | | |
| 20 | 4387 | 8 | <16 | 512 | 256 | 256 | <128 | <128 | 1024 | 1024 | | | | | | |
| 21 | 4471 | 2 | <16 | 512 | 512 | 256 | <128 | <128 | 4096 | 1024 | | | | | | |
| 22 | 4705 | ? | 128 | 2048 | 2048 | 2048 | 128 | <128 | 4096 | 4096 | <40 | 640 | 640 | 640 | | |
| 23 | 5049 | 7 | <16 | 1024 | 512 | 512 | <128 | <128 | 4096 | 1024 | | | | | | |
| 24 | 5065 | 8 | <16 | 2048 | 2048 | 2048 | <128 | <128 | 8192 | 8192 | | | | | | |
| 25 | 5066 | 8 | <16 | 2048 | 1024 | 512 | <128 | <128 | 4096 | 8192 | | | | | | |
| 26 | 5067 | 8 | <16 | 1024 | 512 | 512 | <128 | <128 | 4096 | 1024 | | | | | | |
| 27 | 5293 | 12 | <16 | 4096 | 2048 | 2048 | <128 | <128 | 8192 | 8192 | | | | | | |
| 28 | 5312 | 5 | <16 | 1024 | 1024 | 1024 | <128 | <128 | 4096 | 1024 | | | | | | |
| 29 | 6624 | 4 | <16 | 4096 | 2048 | 2048 | <128 | <128 | 16384 | 4096 | | | | | | |
| 30 | 6625 | 6 | 64 | 2048 | 2048 | 2048 | <128 | <128 | 4096 | 1024 | | | | | | |
| 31 | 6627 | 7 | 64 | 4096 | 1024 | 1024 | <128 | <128 | 8192 | 8192 | | | | | | |
| | 6627-2 | 73 | 64 | 4096 | 512 | 512 | | | | | <40 | 2560 | 320 | 640 | 160 | 80 |
| 32 | 6623 | 13 | 32 | 4096 | 4096 | 2048 | 128 | 128 | 16384 | 8192 | | | | | | |
| 33 | 6626-2 | 108 | 256 | 8192 | 2048 | 2048 | | | | | <40 | 10240 | 1280 | 2560 | 160 | 160 |
| 34 | 6930 | 5 | 256 | 4096 | 2048 | 2048 | <128 | <128 | 8192 | 8192 | | | | | | |
| 35 | 6845 | 4 | <16 | 1024 | 512 | 256 | <128 | <128 | 8192 | 4096 | | | | | | |
| | 6845-a | 29 | <32 | 1024 | 512 | 512 | | | | | <40 | 1280 | 160 | 320 | <40 | <40 |
| | 6855-2 | 11 m | 512 | 4096 | 1024 | 1024 | | | | | <40 | 2560 | 640 | 640 | 80 | 80 |
| 36 | 6931 | 7 | 64 | 2048 | 512 | 512 | <128 | <128 | 4096 | 8192 | | | | | | |
| 37 | 6918 | 13 | <32 | 2048 | 1024 | 512 | <128 | <128 | 8192 | 16384 | | | | | | |
| | 6918a | 27 | <32 | 2048 | 1024 | 512 | | | | | <40 | 2560 | 320 | 1280 | 40 | <40 |
| | 6931-2 | 1y | 512 | 4096 | 1024 | 1024 | | | | | <40 | 5120 | 1280 | 2560 | 160 | 160 |
| 38 | 4481 | 2 m | 256 | 8192 | 8192 | 4096 | <128 | <128 | 2048 | 16384 | | 2560 | 640 | 640 | 160 | 160 |
| 40 | 4482 | 9 | 512 | 8192 | 4096 | 2048 | <128 | <128 | 16384 | 16384 | <40 | 2560 | 2560 | 2560 | <40 | <40 |
| 41 | 4483 | 9 | 1024 | 16384 | 8192 | 8192 | 512 | 256 | 16384 | 16384 | | | | | | |
| 42 | 4484 | 15 | 64 | 8192 | 2048 | 2048 | <128 | <128 | 16384 | 32768 | <40 | 320 | 80 | 80 | <40 | <40 |
| 43 | 4485 | 9 | 128 | 8192 | 4096 | 4096 | 256 | 256 | 32768 | 32768 | | | | | | |
| 44 | | 12 | 512 | 8192 | 4096 | 4096 | 512 | 512 | 16384 | 16384 | | | | | | |
| 45 | 6876 | 6 | <16 | 4096 | 4096 | 4096 | <128 | <128 | 16384 | 8192 | | | | | | |
| 46 | 6878 | 11 | 1024 | 32000 | 4096 | 4096 | 512 | 256 | 32768 | 32768 | | | | | | |
| | 6878-a | 33 | 64 | 8192 | 4096 | 1024 | | | | | <40 | 5120 | 640 | 2560 | 160 | 80 |
| 47 | 6880 | 3 | <16 | 4096 | 2048 | 2048 | <128 | <128 | 4096 | 1024 | | | | | | |
| 48 | 6929 | 4 | <16 | 4096 | 4096 | 4096 | <128 | <128 | 16384 | 1024 | | | | | | |
| 49 | 6628 | 19 | <32 | 2048 | 1024 | 1024 | <128 | <128 | 4096 | 8192 | | | | | | |
| | 6875 | 26 | <32 | 1024 | 512 | 512 | | | | | <40 | 2560 | 640 | 640 | 160 | 160 |
| 51 | 6882 | 17 | 64 | 4096 | 2048 | 2048 | 256 | <128 | 8192 | 8192 | | | | | | |

| Patient no. | No. serum samples | Sampling day after disease onset | IFA | | | | ELISA | | | | FRNT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | PUUV | | DOBV | | | DOBV | | | | |
| | | | PUUV | DOBV | HTNV | SEOV | IgM | IgG | IgM | IgG | PUUV | Sochi | Kurkino | Dobrava | HTNV | SEOV |
| | 6882-a | 34 | 64 | 4096 | 2048 | 1024 | | | | | <40 | 1280 | 320 | 640 | 40 | <40 |
| 52 | 6883 | 13 | 256 | 2048 | 1024 | 1024 | <128 | <128 | 8192 | 8192 | | | | | | |
| | 6883-2 | 10 m | 256 | 4096 | 2048 | 512 | | | | | <40 | 2560 | 80 | 160 | 40 | 40 |
| 53 | 7105 | 10 | 256 | 4096 | 4096 | 2048 | 256 | 128 | 32768 | 32768 | | | | | | |
| 54 | 7110 | 9 | 64 | 1024 | 1024 | 1024 | <128 | <128 | 2048 | 4096 | | | | | | |
| 55 | 7111 | 5 | 64 | 1024 | 1024 | 512 | <128 | <128 | 8192 | 4096 | | | | | | |
| 56 | 8385 | 4 | <16 | 1024 | 512 | 512 | <128 | <128 | 8192 | 2048 | | | | | | |
| 57 | 8386 | 8 | 64 | 1024 | 1024 | 512 | 256 | 128 | 4096 | 8192 | | | | | | |
| 58 | 8380 | 6 | 32 | 2048 | 1024 | 1024 | <128 | <128 | 8192 | 8192 | | | | | | |
| 59 | 8381 | 5 | <16 | 128 | 64 | 64 | <128 | <128 | 1024 | 128 | | | | | | |
| 60 | 8382 | 9 | 32 | 4096 | 4096 | 1024 | <128 | <128 | 32768 | 16384 | | | | | | |
| 61 | 8383 | 16 | <16 | 4096 | 4096 | 2048 | <128 | <128 | 16384 | 16384 | | | | | | |
| 62 | 8384 | 24 | 64 | 4096 | 4096 | 2048 | 256 | 256 | 8192 | 32768 | | | | | | |
| 63 | 8388 | 5 | <16 | 4096 | 2048 | 2048 | <128 | <128 | 8192 | 8192 | | | | | | |

*Patient serum samples were screened for hantavirus antibodies by indirect IFAs by using spot-slides containing a mixture of Vero-E6 cells infected by PUUV, DOBV, HTNV, and SEOV, as previously described (Dzagurova et al., Zh Mikrobiol Epidemiol Immunobiol, 2008; No.1: 12-16). Serum found to be positive was confirmed and further typed on IFA with “monovalent” spot-slides containing cells infected by only 1 of the mentioned viruses. Thereafter, IgG and IgM were determined by ELISAs on the basis of PUUV and DOBV antigens by established methods (Meisel et al., Clin Vaccine Immunol, 2006;13: 1349-57). For further serotyping, the neutralizing activity of serum was investigated by FRNT (Dzagurova et al., Zh Mikrobiol Epidemiol Immunobiol, 2008; No.1: 12-16), using Vero-E6 cells to propagate the following virus stocks; PUUV strain K-27/Ufa-85, DOBV (genotype Sochi) strain Ap1584/Sochi-01 (named Sochi/Ap), DOBV (genotype Kurkino) strain Aa1854/Lipetsk-02, DOBV (genotype Dobrava) strain Bel-1, HTNV strain P-88/ Khabarovsk-89, SEOV strain SR-11. DOBV, Dobrava-Belgrade virus; FRNT, focus-reduction neutralization assay; HTNV, Hantaan virus; IFA, immunofluorescence assay; PUUV, Puumala virus; SEOV, Seoul virus. Blank cells indicate testing not done.

†FRNT was performed with addition of 10% guinea pig blood serum (as complement).

**Technical Appendix Table 2.** Virus sequences and corresponding GenBank accession numbers used in the phylogenetic analyses

| Virus species | Abbreviation | Strain name | GenBank accession no. | |
|---|---|---|---|---|
| | | | S segment | L segment |
| Dobrava-Belgrade virus | DOBV | 10636/Ap | **KP878311** | **KP878308** |
| | | 10645/Ap | **KP878312** | **KP878309** |
| | | 10752/hu | **KP878313** | **KP878310** |
| | | 6882/hu | **–** | **KM192207** |
| | | 43/Ap | JF920151 | **KM192209** |
| | | 79/Ap | JF920152 | **KM192208** |
| | | Sochi/hu | JF920150 | JF920148 |
| | | Sochi/Ap | EU188449 | EU188451 |
| | | GK/Ap | AF442622 | – |
| | | Krasnodar/hu | AF442623 | – |
| | | SK/Aa | AY961615 | GU904039 |
| | | Esl29/Aa | AY533118 | – |
| | | Esl81/Aa | AY533120 | – |
| | | Kurk44/Aa | AJ131672 | – |
| | | Kurk/53/Aa | AJ131673 | – |
| | | Esl856/Aa | AJ269549 | – |
| | | Esl862/Aa | AJ269550 | – |
| | | Lipetsk/Aa | EU188452 | EU188454 |
| | | GER/08/118/Aa | GQ205407 | – |
| | | GER/08/131/Af | GQ205408 | – |
| | | GER/07/293/Aa | GQ205401 | – |
| | | GER/07/607/Af | GQ205402 | – |
| | | GER/07/1064/Aa | GQ205404 | – |
| | | GER/05/239/Aa | GQ205405 | – |
| | | GER/05/477/Af | GQ205406 | – |
| | | Esl400/Af | AY168576 | – |
| | | AP9/Af | AJ410615 | AJ410617 |
| | | AP13/Af | AJ410619 | – |
| | | Slo/Af | L41916 | AJ009779 |
| | | Istanbul/H56 | – | KF039740 |
| | | Saa/160V | AJ009773 | AJ410618 |
| | | Saa/90Aa | AJ009775 | – |
| Sangassou virus | SANGV | SA14 | JQ082300 | JQ082302 |
| | | SA22 | JQ082303 | – |
| Hantaan virus | HTNV | 76–118 | M14626 | NC_005222 |
| | | SC-2 | – | AY675354 |
| | | Z10 | AF184987 | – |
| | | LR1 | AF288294 | – |
| | | AH09 | AF285264 | – |
| | | 84FLi | AY017064 | – |
| Thailand virus | THAIV | 741 | AB186420 | – |
| | | NR/Bi0017 | AM397664 | – |
| Seoul virus | SEOV | 80–39 | NC 005236 | NC_005238 |
| | | Gou3 | AF184988 | – |
| | | Z37 | AF187082 | – |
| | | L99 | AF488708 | – |
| Puumala virus | PUUV | CG1820 | – | M63194 |
| Tula virus | TULV | Mo5302 | – | NC_005226 |
| Andes virus | ANDV | Chile-9717869 | – | NC_003468 |
| Sin Nombre virus | SNV | NM H10 | – | NC_005217 |

*The nucleotide sequences generated in this study were based on PCRs amplifying a 390-nt conserved region within the L segment (Klempa et al. Emerg Infect Dis. 2006;12:838–40) or a 599-nt fragment of the S segment (Klempa et al., J Clin Microbiol, 2005;43:2756–63). Bold type indicates new sequences. Dashes indicate that no sequence information was available.

**Technical Appendix Table 3.** Classification criteria of clinical severity of hemorrhagic fever with renal syndrome*

| Symptoms | Clinical course | | |
|---|---|---|---|
| | Mild | Moderate | Severe |
| Duration of fever, d | 3–4 | 5–6 | >6 |
| Systolic blood pressure, mm Hg | 100 | <100 | <80 |
| Hemorrhagic syndrome | Scleral, subcutaneous hemorrhages | Bleedings, not life threatening | Bleedings, life threatening |
| Duration of oliguria <500 mL/24 h | 24–48 h | 49-96 h | >96 h |
| Anuria >50 mL/24 h | – | – | Positive |
| Serum creatinine, μmol/L | Normal (<96/F, <110/M) | 120–250 | >250 |
| Proteinuria, g/L | <1 | <5 | >5 |
| Pulmonary edema | – | – | Positive |
| Cerebral edema | – | – | Positive |
| Kidney rupture | – | – | Positive |

*Classification according to Russian standard criteria (Leshchinskaia et al., Vopr Virusol. 1990;35:42–5; Klempa et al., Emerg Infect Dis.2008;14:617–25). Dashes indicate that these symptoms were not observed.