

Rio Mamore Hantavirus Endemicity, Peruvian Amazon, 2020

Appendix 1

ELISA procedure according to Anti-Hanta Virus Pool 2 “America” ELISA (IgM), Euroimmun

The samples were diluted in a 1:100 ratio with the dilution buffer containing an anti-human IgG antibody. The mixture was then vortexed and incubated for 10 minutes at room temperature. 100 µl of the calibrator, positive control, negative controls, and diluted patient samples were transferred into the respective microplates. The samples were incubated for 60 minutes at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The microplate was washed three times, with 300 µL of washing buffer. 100 µL of the enzyme conjugate (peroxidase-labeled anti-human IgM) was pipetted into each of the microplate wells and incubated for 30 minutes at room temperature (RT). The microplate was then washed three times as described above. 100 µl of chromogen/substrate solution was pipetted and the microplates were incubated for 15 minutes at RT. Then, 100 µl of stop solution was pipetted into each well. The color intensity was measured photometrically at a wavelength of 450 nm and a reference wavelength between 620 and 650 nm within 30 minutes of adding the stop solution.

ELISA procedure according to Anti-Hanta Virus Pool 2 “America” ELISA (IgG), Euroimmun.

The samples were diluted in a 1:100 ratio with the dilution buffer. 100 µl of the calibrator, positive control, negative controls, and diluted patient samples were transferred into the respective microplates. The incubation and washing procedures remain identical to those previously described. The sole distinction is the utilization of an enzyme conjugate (peroxidase-labeled anti-human IgG).

Calculation of the results

The extinction value of the calibrator establishes the upper limit of the reference range for non-infected individuals (cut-off), as recommended by the manufacturer. Values exceeding the specified cutoff are considered positive, while those below the cutoff are considered negative. The ratio is calculated according to the following formula:

$$(\text{Extinction of the control or patient sample})/(\text{Extinction of calibrator}) = \text{Ratio}$$

The ratio results were interpreted as follows:

Ratio <0.8: Negative

Ratio \geq 0.8 to <1.1: Borderline

Ratio \geq 1.1: Positive

Reference

1. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol.* 2018;35(6):1547–9.

Appendix Table 1. List of primers used in this study

| Primer name | Primer sequence | Orientation |
|------------------|-------------------------------------|-------------|
| S segment | | |
| RM_1FO | GAAGGTATCACAAATCCACGAGCAGCA | Forward |
| RM_1FI | AGTGGAGGTGGACCCAGATGACGT | Forward |
| RM_1R | CTTCATTGTGGATTGTGCTGTTGGCA | Reverse |
| RM_2FO | CCAACAGCACAATCCACAATGAAGGC | Forward |
| RM_2F1 | AAGGCGGATGAAATTACACCAGGAAGA | Forward |
| RM_2R | AGCTGACTCTGACTCTGCTGCA | Reverse |
| RM_3F | CAAGCAGCAGAGTCAGAGTCAGC | Forward |
| RM_3RI | GGATTGTGCTAGCTGCCTGAGC | Reverse |
| RM_3RO | CCTTCGCATCTATCAGGGATTGTGC | Reverse |
| RM_4F | GCTAGCACAATCCCTGATAGATGCG | Forward |
| RM_4RI | CACAGTCAATGTTAGTGTTCATAGC | Reverse |
| RM_4RO | GAGGTAGAAATGTGCACAGTCAATGTTAGTG | Reverse |
| M segment | | |
| RM_5FO | TGCAACTTTGATCTCCATACCACCG | Forward |
| RM_5FI | GTGACACAACCTGACACCACAGAGGC | Forward |
| RM_5R | CTTGAATAGCATTATCAGTACAACCTG | Reverse |
| RM_6F | AAACAGGTTGACTGATAATGCTATTCAAG | Forward |
| RM_6RI | CTCCTGATATTGGCAGATACCCAGTCC | Reverse |
| RM_6RO | AATAAGCCTCACAACTTGCTCCAGG | Reverse |
| RM_7F | GCCCTGGAGCAAGTTGTGAGGC | Forward |
| RM_7RI | ACTCCAAGTGTACGGTAACATCCTCG | Reverse |
| RM_7RO | GCATCTACTCCTATACCAGGAACACTCC | Reverse |
| RM_8F | CACTTGGAGTGTTCCGGTATAGGAGTAG | Forward |
| RM_8RI | CAGTACATCCAGTTC AACACCAGGAC | Reverse |
| RM_8RO | ACCCCGCATGCAGTACATCCAGT | Reverse |
| RM_9FO | GCGATTGTCTGGTGTGGAAGTGG | Forward |
| RM_9FI | TGGAAGTGGATGACTGCATGCGG | Forward |
| RM_9R | ACACTTAAACGTGCATGGAGGTGCC | Reverse |
| RM_10FO | GGCACCTCCATGCACGTTTAAAGTG | Forward |
| RM_10FI | CTCCATGCACGTTTAAAGTGTGGTTCAC | Forward |
| RM_10R | GTATGCTCCGCAGGAACAAAAGCCT | Reverse |
| L segment | | |
| RM_11F | AGTAGTAGACTCCGGGATAGAAAAGATCAG | Forward |
| RM_11RI | GTATCACACTGGTCTGAACTAATAGTAGAGG | Reverse |
| RM_11RO | ATCTCTAAACAGCCGCTTGTATCAC | Reverse |
| RM_12FO | TACTATTAGTTCAGACCAGTGTGATACAAGGA | Forward |
| RM_12FI | GTTCCAGACCAGTGTGATACAAGGATCCGG | Forward |
| RM_12R | ACTACCAATGTTGATACTTTCAATCATCTCACC | Reverse |
| RM_13F | GGTGAGATGATTGAAAGTATCAACATTGGTAG | Forward |
| RM_13RI | CTCCTACTGTTGAATGATCTACTGTGAGAC | Reverse |
| RM_13RO | TATACTACTCTAGACATAAGTGAAGGATAGACC | Reverse |
| RM_14FO | CTCACAGTAGATCATTCAACAGTAGGAGC | Forward |
| RM_14FI | GGGTCTATCCTTCACTTATGTCTAGAGTAG | Forward |
| RM_14R | GATGACCCTGATGCCCATCTTAAAGC | Reverse |
| RM_15F | AGAGAAAGCTTTAAGATGGGCATCAGG | Forward |
| RM_15RI | TTACATTTGCTGACACGCCACCAG | Reverse |
| RM_15RO | AATCCCTTGACGCCAATTACCTTTTAC | Reverse |
| RM_16FO | TTCTGGTGGCGTGTGCAAGCAATGTA | Forward |
| RM_16FI | GTTTCATCATTATTCGGCTCAGCTATTTCC | Forward |
| RM_16R | TGCATAGTCATTGCAGTGACAATACTAGG | Reverse |
| RM_17FO | AAACTGAATGATCCTAGTATTGCTACTGCA | Forward |
| RM_17FI | TGACTATGCAGTCACCGTTACAATTACG | Forward |
| RM_17RI | GATCATTGCTTTGCACCACCTCCA | Reverse |
| RM_17RO | GCATTGAGCCACTCTCTTACATCCTG | Reverse |
| RM_18F | TATTGAAGTATGGAGGTGGTGCAAAGCA | Forward |
| RM_18RI | GTCACCCCTTAGATCACCCCTCATAACAGC | Reverse |
| RM_18RO | TCAAGGCGTGCACAATCAAGACCA | Reverse |
| RM_19FO | AGAATTGATAAGATGTGGTATGAGATTCAAACCTG | Forward |
| RM_19FI | CCAAACACGGAGTCTTGTATTATTCAAGACA | Forward |
| RM_19R | GTTGAATRCCTTTCTCTAACAAGTAACTGTG | Reverse |
| RM_20FO | GGAYAGAGAAGCACAGTTACTTGTAGAGA | Forward |
| RM_20FI | CACAGTACTTGTAGAGAGAAAGGYATTCAAC | Forward |
| RM_20R | TAGTAGTATGCTCCGGGAAAAGAACT | Reverse |

Complementary DNA was generated using SuperScriptIII reverse transcriptase and random hexamer primers followed by PCR using the Platinum™ Taq DNA Polymerase Kit, according to the manufacturer's instructions (<https://www.thermofisher.com>) and the following cycling conditions: 95°C for 3 min, followed by 45 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min with a final extension of 72°C 10 min. Products were purified using a GenUP™ ExoSAP kit (<https://www.biotechrabbit.com/>) and subsequently subjected to Sanger sequencing. O: primer used in the first round I: primer used in the second round.

Appendix Table 2. Primers and probe for qRT-PCR

| Primer name | Sequence | Orientation |
|-------------|---|-------------|
| RIOMV F | AGGCAATCTATGATGGATTATCTG | Forward |
| RIOMV R | CAATCGTTCATCTCATCTATATACC | Reverse |
| RIOMV Probe | 6FAM-TGGATGCACTTCGGAATATATATGAAACAG-IABKFQ3 | Probe |

The qRT-PCR reaction was carried out using the SuperScript III One-Step RT-PCR with Platinum Taq DNA Polymerase Kit (<https://www.thermofisher.com>) according to the manufacturer's instructions. The qRT-PCR was performed on a Roche LightCycler® 480 II with the following conditions: a reverse transcription reaction for 20 min at 55°C, followed by denaturation at 95°C for 3 min, followed by 45 cycles of 95°C for 15 sec, and 55°C for 30 sec. The viral load was calculated using a standard curve relying on a photometrically quantified in vitro-transcribed RNA control. FAM: Fluorescein amidites, IABKFQ: 3' Iowa Black® fluorescent quencher.

Appendix Table 3. Reference sequences used for phylogenetic tree inferences

| Virus | Virus name abbreviation | Strain | L | M | S | Host detected | Country |
|-------------------------|-------------------------|-----------------------------------|----------|----------|----------|----------------------------------|--------------------------------|
| Alto Paraguay virus | ALPA | A1Pa | NA | NA | DQ345762 | <i>Holochilus chacarius</i> | Paraguay (PRY) |
| Andes virus | ANDV | Chile-9717869 | AF291704 | AF291703 | AF291702 | NA | Chile (CHI) |
| Araucaria virus | ARAUV | HPR/03-95 | NA | NA | AY740628 | NA | Brazil (BRA) |
| Asama virus | ASAV | N10 | NA | EU929075 | EU929072 | <i>Urotrichus talpoides</i> | Japan (JPN) |
| Asikkala virus | ASIV | CZ/Beskydy/4 12/2010/Sm | KC880347 | KC880344 | KC880341 | <i>Sorex minutus</i> | Czech Republic (CZE) |
| Bayou virus | BAYV | HV F0260003 | GQ244526 | L36930 | L36929 | <i>Homo sapiens</i> | United States of America (USA) |
| Bermejo virus | BMJV | Oc22531 | NA | NA | AF482713 | <i>Oligoryzomys chacoensis</i> | Argentina (ARG) |
| Black Creek Canal virus | BCCV | NA | L39951 | L39950 | L39949 | NA | United States of America (USA) |
| Bowé virus | BOWV | VN1512 | KC631784 | KC631783 | KC631782 | <i>Crociodura douceti</i> | Guinea (GIN) |
| Bruges virus | BRUV | BE/ieux/Gen appe/TE/2013/ 1 | KX551962 | KX551961 | KX551960 | <i>Talpa europea</i> | Belgium (BEL) |
| Caño Delgadito virus | CADV | VHV-574 | GQ200821 | DQ284451 | DQ285566 | <i>Sigmodon alstoni</i> | Venezuela (VEN) |
| Cao Bang virus | CBV | 3 | EF543525 | EF543526 | EF543524 | <i>Anourosorex squamipes</i> | Vietnam (VNM) |
| Carrizal virus | CARV | 2/2006 | NA | NA | AB620093 | <i>Peromyscus megalops</i> | Mexico (MEX) |
| Catacamas virus | CATV | HV C1280001 | NA | NA | DQ256126 | <i>Oryzomys couesi</i> | Honduras (HND) |
| Choclo virus | CHOV | MSB96073 | NA | DQ285047 | DQ285046 | <i>Oligoryzomys fulvescens</i> | Panama (PAN) |
| Convict Creek virus | CCV | Convict creek 74 | NA | NA | L33816 | NA | United States of America (USA) |
| Dabieshan virus | DABV | Yongjia-Nc-58 | NA | JF796036 | JF796022 | <i>Niviventer confucianus</i> | China (CHN) |
| Dobrava-Belgrade virus | DOBV | Ano/Poroia/Afl 9/1999 | AJ410617 | AJ410616 | AJ410615 | <i>Apodemus flavicollis</i> | Greece (GRC) |
| El Moro Canyon virus | ELMCV | RM-97 | NA | U26828 | U11427 | <i>Reithrodontomys megalotis</i> | Mexico (MEX) |
| Fugong virus | FUGV | FG10 | KT899703 | KT899702 | KT899701 | <i>Eothenomys eleusis</i> | China (CHN) |
| Hantaan virus | HTNV | 76-118 | X55901 | M14627 | M14626 | NA | Republic of Korea (KOR) |
| Jeju virus | JEJV | 43779 | HQ834697 | HQ834696 | HQ834695 | <i>Crociodura shantungensis</i> | Republic of Korea (KOR) |
| Kenkeme virus | KENV | Fuyuan-Sr- 326 | KJ857320 | KJ857337 | KJ857341 | <i>Sorex roboratus</i> | China (CHN) |
| Khabarovsk virus | KHAV | Fuyuan-Mm- 217 | KJ857321 | KJ857338 | KJ857342 | <i>Microtus maximowiczii</i> | China (CHN) |
| Laguna Negra virus | LANV | 510B | NA | AF005728 | AF005727 | <i>Calomys laucha</i> | Paraguay (PRY) |
| Lechiguanas virus | LECV | 22819 | NA | NA | AF482714 | <i>Oligoryzomys flavescens</i> | Argentina (ARG) |
| Luxi virus | LUXV | LX309 | HQ404253 | HM756287 | HM756286 | <i>Eothenomys miletus</i> | China (CHN) |
| Maciel virus | MACV | 13796 | NA | NA | AF482716 | <i>Necomys benefactus</i> | Argentina (ARG) |

| Virus | Virus name abbreviation | Strain | L | M | S | Host detected | Country |
|---------------------|-------------------------|------------------|------------|------------|------------|----------------------------------|--------------------------------|
| Maporal virus | MAPV | HV 97021050 | EU788002 | AY363179 | AY267347 | <i>Oligoryzomys fulvescens</i> | Venezuela (VEN) |
| Maripa virus | MARV | BOR | JQ611713.1 | JQ611714.1 | JQ611712.1 | <i>Homo sapiens</i> | French Guiana (GUY) |
| Montano virus | MTNV | 104/2006 | AB620102 | AB620101 | AB620100 | <i>Peromyscus beatae</i> | Mexico (MEX) |
| Necocli virus | NECV | HV O0020002 | KF735065 | KF494345 | KF481954 | <i>Zygodontomys brevicauda</i> | Colombia (COL) |
| New York virus | NYV | RI-I | NA | NA | UO9488 | <i>Homo sapiens</i> | United States of America (USA) |
| Oxbow virus | OXBV | Ng1453 | FJ593497 | FJ539167 | FJ539166 | <i>Neurotrichus gibbsii</i> | United States of America (USA) |
| Pergamino virus | PERV | 14403 | NA | NA | AF482717 | NA | Argentina (ARG) |
| Prospect Hill virus | PHV | PH-1 | EF646763 | X55129 | Z49098 | NA | United States of America (USA) |
| Puumala virus | PUUV | CG1820 | M63194 | M29979 | M32750 | NA | Finland (FIN) |
| Rio Mamore virus | RIOMV | OM-556 | NA | NA | U52136 | <i>Oligoryzomys microtis</i> | Bolivia (BOL) |
| Rio Mamore virus | RIOMV | AN683313/BR A271 | NA | NA | JX443667 | <i>Oligoryzomys microtis</i> | Brazil (BRA) |
| Rio Mamore virus | RIOMV | AN693292/BR A293 | JX443697 | JX443700 | JX443679 | <i>Oligoryzomys sp</i> | Brazil (BRA) |
| Rio Mamore virus | RIOMV | LH 060/2011 | NA | NA | KF584259 | <i>Homo sapiens</i> | Brazil (BRA) |
| Rio Mamore virus | RIOMV | HTN-007 | FJ809772 | FJ608550 | FJ532244 | <i>Oligoryzomys microtis</i> | Peru (PER) |
| Rio Segundo virus | RIOSV | RMx-Costa-1 | NA | NA | U18100 | <i>Reithrodontomys mexicanus</i> | Costa Rica (CRI) |
| Rockport virus | ROCV | MSB57412 | HM015221 | HM015219 | M015223 | <i>Scalopus aquaticus</i> | United States of America (USA) |
| Sangassou virus | SANGV | SA14 | JQ082302 | JQ082301 | JQ082300 | <i>Hylomyscus simus</i> | Guinea (GIN) |
| Seoul virus | SEOV | 80-39 | X56492 | S47716 | AY273791 | <i>Rattus norvegicus</i> | Republic of Korea (KOR) |
| Sin Nombre virus | SNV | NM R11 | L37902 | L37903 | L37904 | <i>Peromyscus maniculatus</i> | United States of America (USA) |
| Tula virus | TULV | Moravia/5302v | AJ005637 | Z69993 | Z49915 | NA | Russia (RUS) |

NA: Not available.

Appendix Table 4. Reference sequences used for phylogenetic tree inferences of the Laguna Negra clade

| Name | Accession no. | Virus | Country | Host |
|----------------------------|---------------|---------------------|---------------|--------------------------------|
| S segment | | | | |
| ALPA Aipa PRY | DQ345762.1 | Alto Paraguay virus | Paraguay | <i>Holochilus chacarius</i> |
| ANAJV AN669104 BRA 2003 | HM238886.1 | Anajatuba virus | Brazil | <i>Necromys lasiurus</i> |
| ANAJV AN690936 BRA 2003 | HM238887.1 | Anajatuba virus | Brazil | <i>Oligoryzomys fornesi</i> |
| ANAJV AN690985 BRA 2003 | HM238888.1 | Anajatuba virus | Brazil | <i>Oligoryzomys fornesi</i> |
| ANAJV H666379 BRA | HM238889.1 | Anajatuba virus | Brazil | <i>Homo sapiens</i> |
| ANAJV H670957 | HM238890.1 | Anajatuba virus | Brazil | <i>Homo sapiens</i> |
| ANAJV H672862 BRA 2003 | HM238885.1 | Anajatuba virus | Brazil | <i>Homo sapiens</i> |
| ANAJV Of58 | DQ451829.1 | Anajatuba virus | NA | <i>Oligoryzomys fornesi</i> |
| LANV | AF005727.1 | Laguna Negra virus | NA | <i>Calomys laucha</i> |
| LANV AN649993 BRA 2001 | JQ775501.1 | Laguna Negra virus | Brazil | <i>Callomys callidus</i> |
| LANV AN650204 BRA 2001 | JQ775500.1 | Laguna Negra virus | Brazil | <i>Callomys callidus</i> |
| LANV AN650228 BRA 2001 | JQ775502.1 | Laguna Negra virus | Brazil | <i>Callomys callidus</i> |
| LANV H650736 BRA 2001 | JQ775504.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H651686 BRA 2002 | JQ775511.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H653486 BRA 2002 | JQ775514.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H657848 BRA 2002 | JQ775517.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H660462 BRA 2002 | JQ775506.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H671696 BRA 2003 | JQ775505.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H678213 BRA 2005 | JQ775513.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H682807 BRA 2004 | JQ775507.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H695325 BRA 2005 | JQ775515.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H695689 BRA 2005 | JQ775508.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H696558 BRA 2005 | JQ775512.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H706738 BRA 2006 | JQ775516.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H710031 BRA 2006 | JQ775510.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H712518 BRA 2006 | JQ775518.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV H713175 BRA 2006 | JQ775509.1 | Laguna Negra virus | Brazil | <i>Homo sapiens</i> |
| LANV LBCE 12234 BRA 2010 | KP202359.1 | Laguna Negra virus | Brazil | <i>Calomys callidus</i> |
| MARV BOR GUY 2009 | JQ611712.1 | Maripa virus | French Guiana | NA |
| MARV DOS GUY 2017 | MG785209.1 | Maripa virus | French Guiana | <i>Homo sapiens</i> |
| MARV F0102 GUY 2010 | JN982967.1 | Maripa virus | French Guiana | <i>Oligoryzomys fulvescens</i> |
| MARV GOU GUY 2008 | KC876041.1 | Maripa virus | French Guiana | <i>Homo sapiens</i> |
| MARV KES GUY 2013 | MF101865.1 | Maripa virus | French Guiana | <i>Homo sapiens</i> |
| MARV RAD GUY 2010 | MF101864.1 | Maripa virus | French Guiana | <i>Homo sapiens</i> |
| MARV VANG GUY 2016 | MF101866.1 | Maripa virus | French Guiana | <i>Homo sapiens</i> |
| RIOMM Hs85 ARG | DQ451828.1 | Rio Mearim virus | NA | <i>Holochilus sciureus</i> |
| RIOMV 2020 PER | ERR11860590 | Rio Mamore virus | Peru | <i>Homo sapiens</i> |
| RIOMV BRA 2004 | JX443667.1 | Rio Mamore virus | Brazil | <i>Oligoryzomys microtis</i> |
| RIOMV BRA 2005 | JX443679.1 | Rio Mamore virus | Brazil | <i>Oligoryzomys sp.</i> |
| RIOMV HTN-007 PER 1996 | FJ532244.1 | Rio Mamore virus | Peru | <i>Oligoryzomys microtis</i> |
| RIOMV LH 060/2011 BRA 2011 | KF584259.1 | Rio Mamore virus | Brazil | <i>Homo sapiens</i> |
| RIOMV OM-137 BOL | U52137.1 | Rio Mamore virus | Bolivia | <i>Oligoryzomys microtis</i> |
| RIOMV OM-142 BOL | U52138.1 | Rio Mamore virus | Bolivia | <i>Oligoryzomys microtis</i> |
| RIOMV OM-143 BOL | U52139.1 | Rio Mamore virus | Bolivia | NA |
| RIOMV OM-556 BOL 1985 | U52136.1 | Rio Mamore virus | Bolivia | <i>Oligoryzomys microtis</i> |
| RIOMV OM-604 BOL | U52140.1 | Rio Mamore virus | Bolivia | NA |
| M segment | | | | |
| RIOMV HTN-007 PER 1996 | FJ608550 | Rio Mamore virus | Peru | <i>Oligoryzomys microtis</i> |
| ALPA Aipa PRY | AY515602 | Alto Paraguay virus | Paraguay | <i>Holochilus chacarius</i> |
| LANV | NC_038506 | Laguna Negra virus | NA | <i>Calomys laucha</i> |
| LANV AN29582 ARG 2000 | JX891631 | Laguna Negra virus | Argentina | <i>Calomys callosus</i> |
| MARV BOR GUY 2009 | JQ611714.1 | Maripa virus | French Guiana | <i>Homo sapiens</i> |
| RIOMV BRA 2004 | JX443701.1 | Rio Mamore virus | Brazil | <i>Oligoryzomys microtis</i> |
| RIOMV BRA 2005 | JX443700.1 | Rio Mamore virus | Brazil | <i>Oligoryzomys sp.</i> |
| RIOMV OM-137 BOL | U73687 | Rio Mamore virus | Bolivia | <i>Oligoryzomys microtis</i> |
| RIOMV OM-556 BOL 1985 | U73688 | Rio Mamore virus | Bolivia | <i>Oligoryzomys microtis</i> |

NA: Not available.

Appendix Table 5. Amino acid distance matrix for complete nucleoprotein sequences using a p-distance substitution model. The final data set contained 433 positions for the nucleoprotein, analyses were conducted in MEGA X (1)

| Virus | RIOMV | MARV | LANV | ANDV | SNV | DOBV | HTNV | PUUV | SAAV | SEOV |
|-------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|------|
| RIOMV PER 1996 FJ532244 | | | | | | | | | | |
| MARV GUY KC876041 | 0.0280 | | | | | | | | | |
| LANV PRY NC_038505 | 0.0724 | 0.0724 | | | | | | | | |
| ANDV CHI NC_003466 | 0.0888 | 0.0864 | 0.0958 | | | | | | | |
| SNV USA L37904 | 0.1542 | 0.1495 | 0.1472 | 0.1402 | | | | | | |
| DOBV GRC AJ410615 | 0.3668 | 0.3645 | 0.3598 | 0.3528 | 0.3715 | | | | | |
| HTNV KOR M14626 | 0.3528 | 0.3528 | 0.3551 | 0.3481 | 0.3715 | 0.1702 | | | | |
| PUUV FIN NC_005224 | 0.2757 | 0.2757 | 0.2757 | 0.2710 | 0.2921 | 0.3846 | 0.3893 | | | |
| SAAV DEN AJ616854 | 0.3738 | 0.3715 | 0.3668 | 0.3598 | 0.3738 | 0.0140 | 0.1655 | 0.3846 | | |
| SEOV KOR AY273791 | 0.3621 | 0.3551 | 0.3621 | 0.3528 | 0.3762 | 0.1841 | 0.1678 | 0.3753 | 0.1772 | |

Appendix Table 6. Amino acid distance matrix for partial nucleoprotein sequences using a p-distance model. The final data set contained 276 positions for the nucleoprotein, analyses were conducted in MEGA X (7)

| Virus | RIOMV 2020 | RIOMV 1996 | MAPV | LANV | ANDV | SNV |
|-------------------------|------------|------------|--------|--------|--------|-----|
| RIOMV 2020 | | | | | | |
| RIOMV PER 1996 FJ532244 | 0.0000 | | | | | |
| MARV GUY KC876041 | 0.0291 | 0.0291 | | | | |
| LANV PRY NC_038505 | 0.0436 | 0.0436 | 0.0509 | | | |
| ANDV CHI NC_003466 | 0.0655 | 0.0655 | 0.0691 | 0.0655 | | |
| SNV USA L37904 | 0.1200 | 0.1200 | 0.1164 | 0.1055 | 0.1055 | |

Appendix Table 7. Amino acid distance matrix for glycoprotein sequences using a p-distance model. The final data set contained 1139 positions for the glycoprotein, analyses were conducted in MEGA X (7). Saaremaa virus was not included in the study due to the absence of a complete M sequence.

| Virus | RIOMV | MARV | LANV | ANDV | SNV | DOBV | HTNV | PUUV | SEOV |
|-------------------------|--------|--------|--------|--------|--------|--------|--------|--------|------|
| RIOMV PER 1996 FJ608550 | | | | | | | | | |
| MARV GUY JQ611714 | 0.0378 | | | | | | | | |
| LANV PRY AF005728 | 0.0879 | 0.0914 | | | | | | | |
| ANDV CHI NC_003467 | 0.1230 | 0.1221 | 0.1353 | | | | | | |
| SNV USA L37903 | 0.2250 | 0.2293 | 0.2320 | 0.2206 | | | | | |
| DOBV GRC AJ410616 | 0.4527 | 0.4500 | 0.4589 | 0.4580 | 0.4589 | | | | |
| HTNV KOR M14627 | 0.4466 | 0.4457 | 0.4537 | 0.4501 | 0.4501 | 0.2259 | | | |
| PUUV FIN M29979 | 0.3369 | 0.3404 | 0.3456 | 0.3369 | 0.3275 | 0.4660 | 0.4625 | | |
| SEOV KOR NC_005237 | 0.4571 | 0.4571 | 0.4598 | 0.4571 | 0.4686 | 0.2277 | 0.2286 | 0.4633 | |

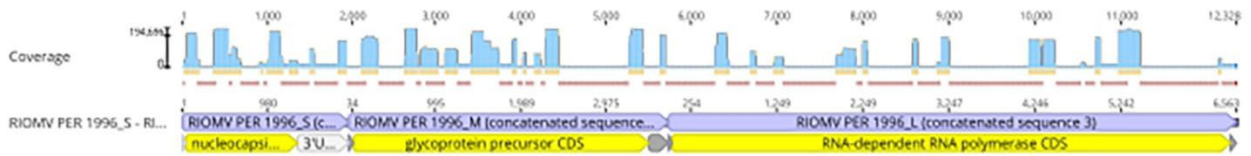
Appendix Table 8. Comparison of IgG ELISA reactivity in complementary testing

| Type of ELISA/ Group | Ecozone | | | |
|----------------------|---------------------------------------|-------------|-------------|-------------|
| | Lambayeque | Lima | Loreto | |
| CHIKV | Hantavirus IgG ELISA-positive samples | 0% | 0% | 25% (n=2) |
| | Control group | 0% | 0% | 7.7% (n=1) |
| | Total | 0% | 0% | 14.3% (n=3) |
| MAYV | Hantavirus IgG ELISA-positive samples | 0% | 0% | 37.5% (n=3) |
| | Control group | 0% | 15.4% (n=2) | 7.7% (n=1) |
| | Total* | 0% | 11.8% (n=2) | 19.0% (n=4) |
| OROV | Hantavirus IgG ELISA-positive samples | 0% | 0% | 12.5% (n=1) |
| | Control group | 0% | 0% | 38.5% (n=5) |
| | Total | 0% | 0% | 28.6% (n=6) |
| Plasmodium | Hantavirus IgG ELISA-positive samples | 62.5% (n=5) | 75% (n=3) | 37.5% (n=3) |
| | Control group | 8.3% (n=1) | 30.8% (n=4) | 7.7% (n=1) |
| | Total† | 30% (n=6) | 41.2% (n=7) | 20% (n=4) |
| SARS-CoV-2 | Hantavirus IgG ELISA-positive samples | 0% | 0% | 12.5% (n=1) |
| | Control group | 8.3% (n=1) | 0% | 7.7% (n=1) |
| | Total‡ | 5% (n=1) | 0% | 10% (n=2) |

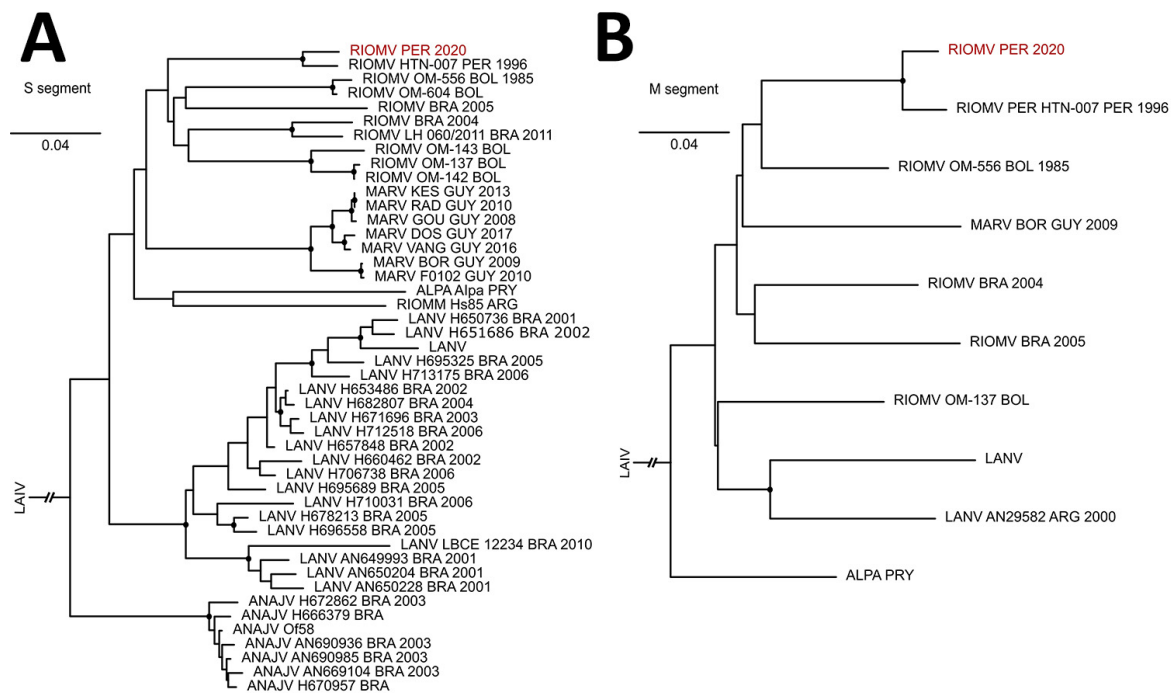
CHIKV: Chikungunya virus, MAYV: Mayaro virus, OROV: Oropouche virus, SARS-CoV-2 S: Severe acute respiratory syndrome coronavirus 2. The results excluded the two sera that tested positive for cytomegalovirus. Hantavirus IgG ELISA-positive samples: Lambayeque n=8, Lima n=4, and Loreto n=8. Control group: Lambayeque n=12, Lima n=13 and Loreto n=13. Cells with zero-cell counts were excluded from the statistical analysis.

*Total samples from Lima and Loreto were compared by a Fisher's exact test: $p=0.7$;

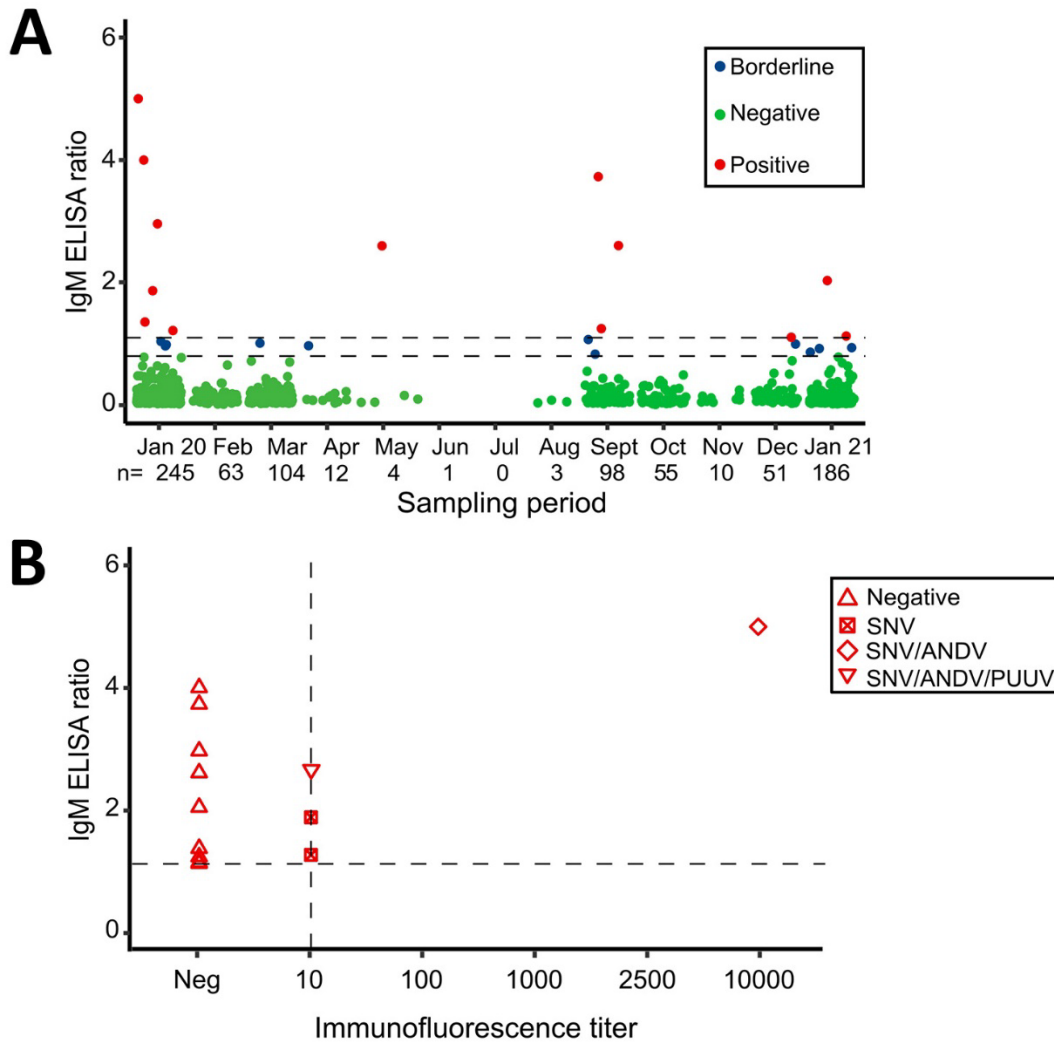
†: Total samples from Lambayeque, Lima, and Loreto were compared by a Chi-Square test: $X^2=2.2$, $p=0.33$; ‡: Total samples from Lambayeque, and Loreto were compared by a Fisher's exact test: $p=0.2$.



Appendix Figure 1. Coverage of Rio Mamore 2020 (RIOMV 2020) segments compared to Rio Mamore 1996 (RIOMV 1996) strain. The partial sequences obtained from high-throughput and Sanger sequencing are shown in orange. The alignment was constructed using Geneious (2023.2.1). The nucleotide positions are indicated by segment.



Appendix Figure 2. Phylogenetic relationships between the Rio Mamore virus from Peru (RIOMV PER 2020, shown in red) and *Orthohantavirus mamorense* species. A) Partial sequence of the S segment (276 nt). B) Partial sequence of the M segment (150 nt). Phylogenetic trees were constructed using a Neighbor-joining (NJ) method with 1000 bootstraps and p-distance. The percentage of replicate trees in which the associated taxa clustered ≥ 0.80 in the bootstrap test is marked as a black dot. The location, year, and strain are indicated when available. Due to the low overlap of partial genome sequences available in GenBank, resulting in a small dataset of limited phylogenetic information, simplistic NJ trees were used instead of probabilistic methods as used for larger genomic sequences.



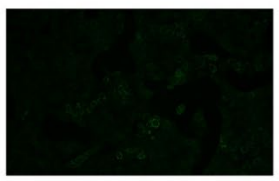
Appendix Figure 3. Hantavirus incidence in the Peruvian amazon, 2020–2021. A) Andes virus (ANDV) and Sin Nombre virus (SNV) IgM ELISA reactivities in Loreto, Peru, between January 2020 and January 2021, single study datum is indicated as a dot. B) ANDV and SNV positive serum samples in IgM ELISA compared to IgM immunofluorescence assay (IFA) titers.

A

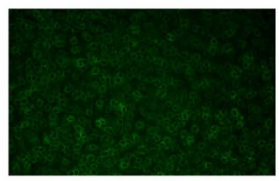
IgM samples

1395

1:10 SNV

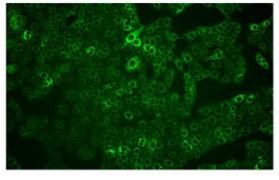


1:10 ANDV

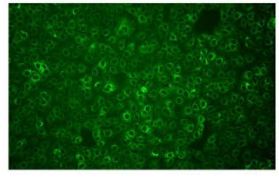


3260

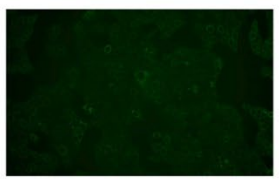
1:10 SNV



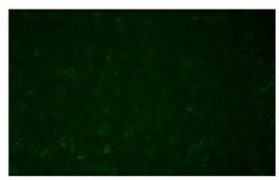
1:10 ANDV



1:10000 SNV

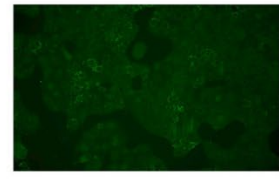


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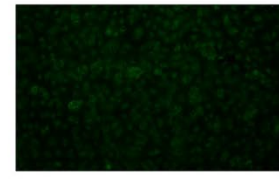


3376

1:10 SNV

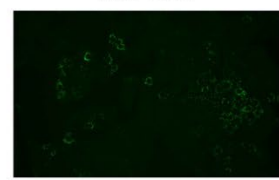


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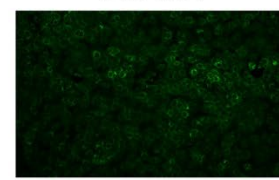


4242

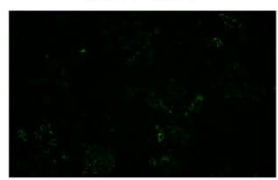
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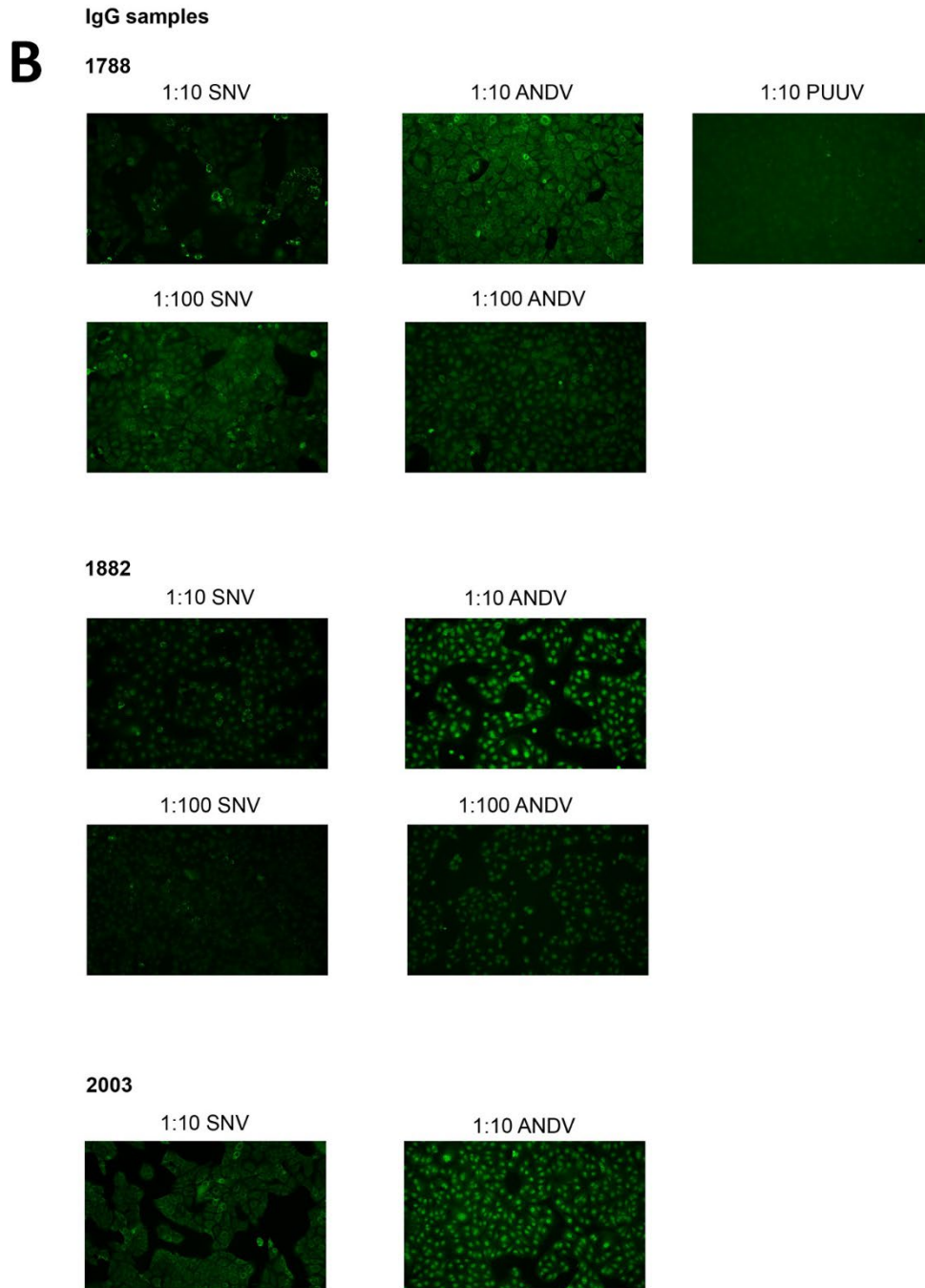


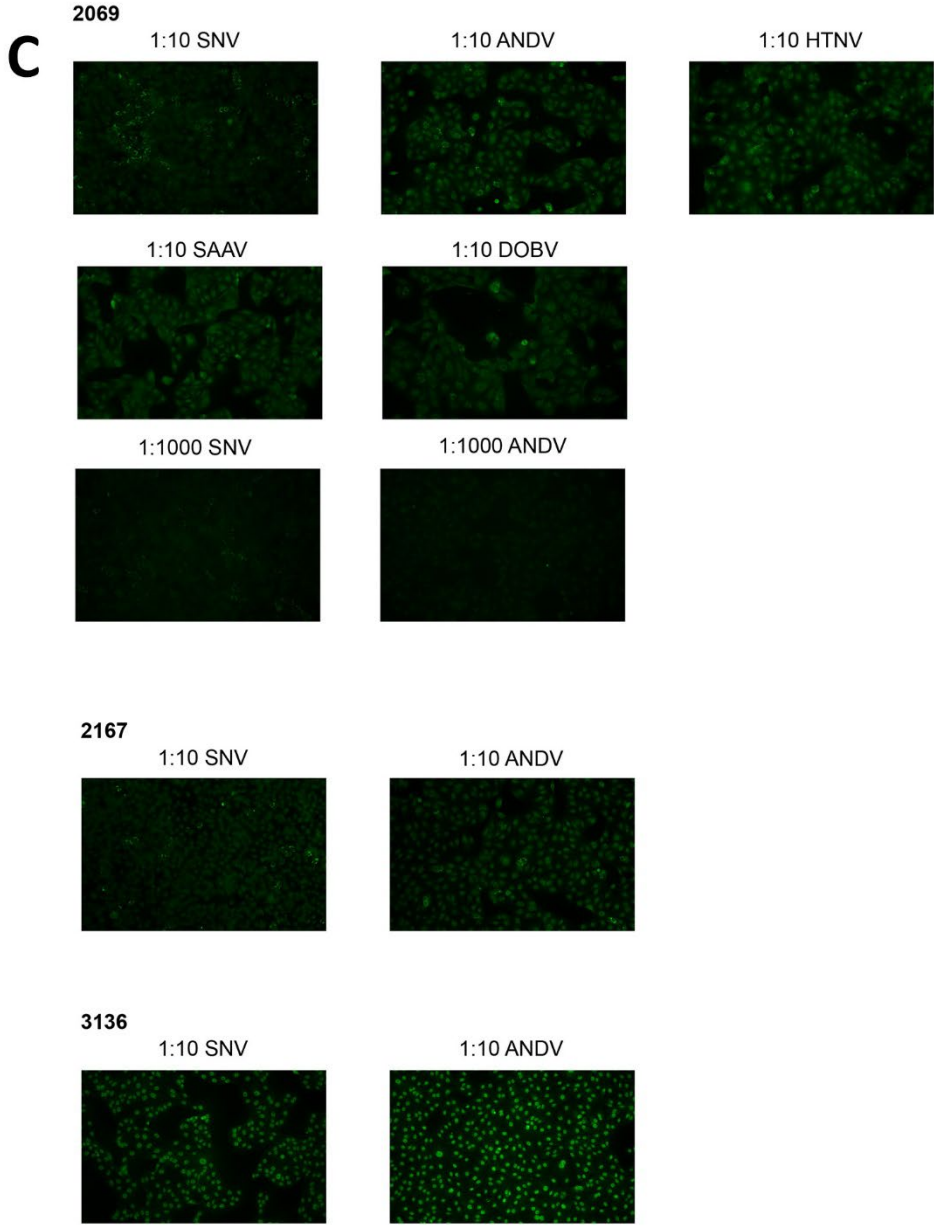
1:10 ANDV



1:10 PUUV



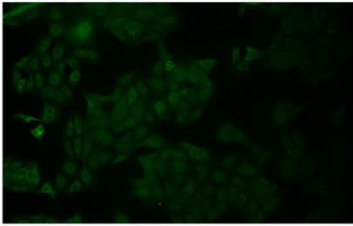




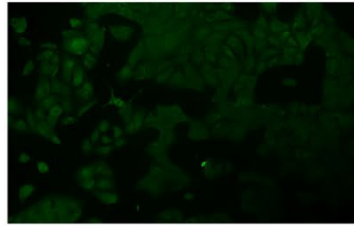
D

3249

1:10 DOBV

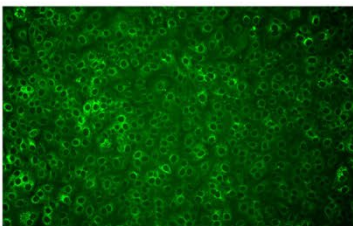


1:10 SAAV

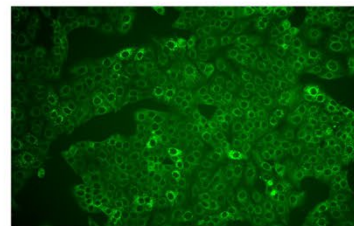


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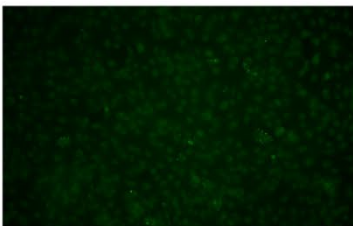
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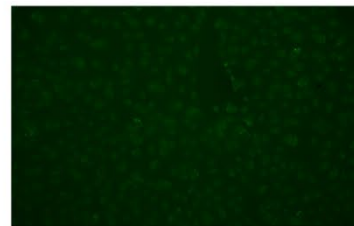
1:10 ANDV



1:10000 SNV

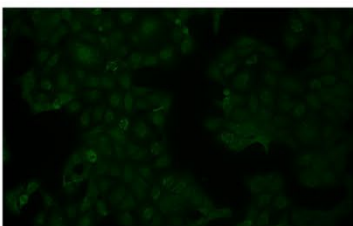


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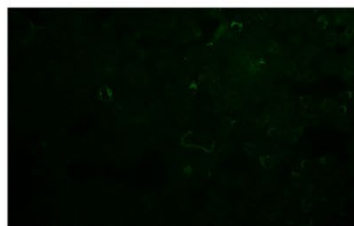


3317

1:10 DOBV

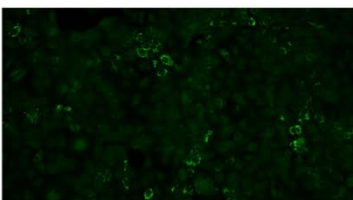


1:10 HTNV

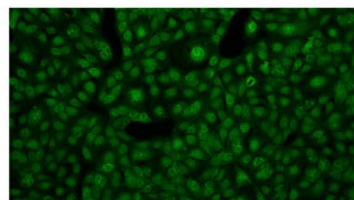


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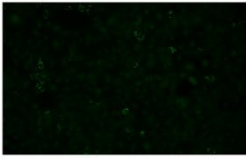


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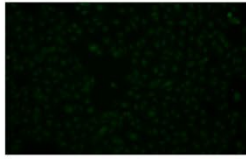


E

1:100 SNV

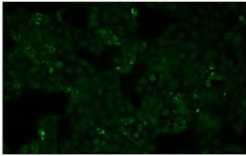


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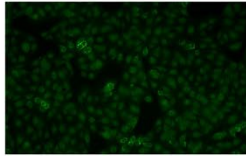


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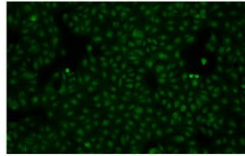
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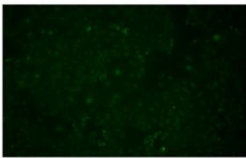
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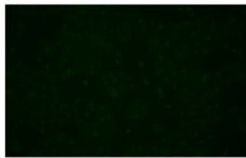
1:10 PUUV



1:2500 SNV

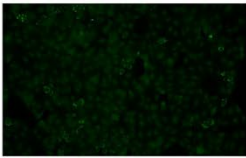


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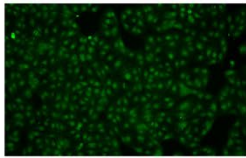


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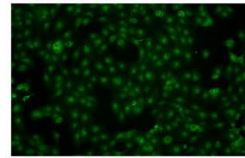
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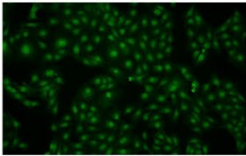
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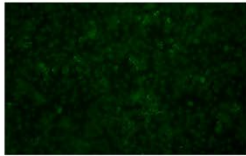
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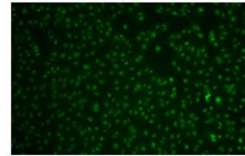
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1:100 SNV



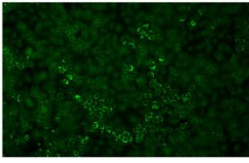
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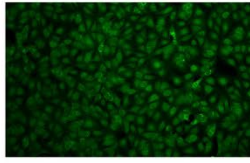
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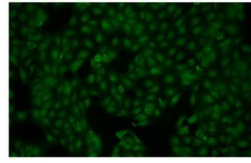
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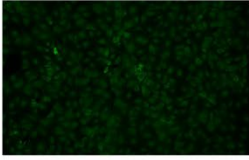
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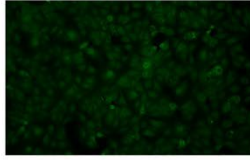
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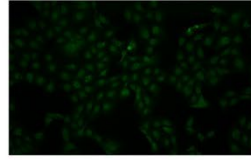
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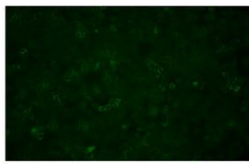
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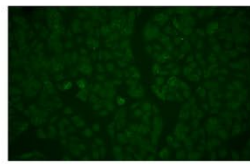
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1:10000 SNV

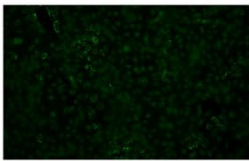


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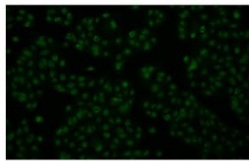


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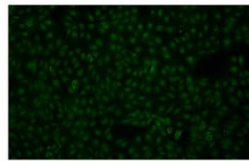
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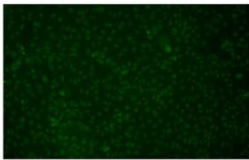
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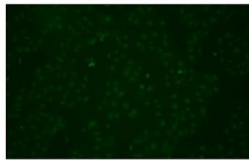
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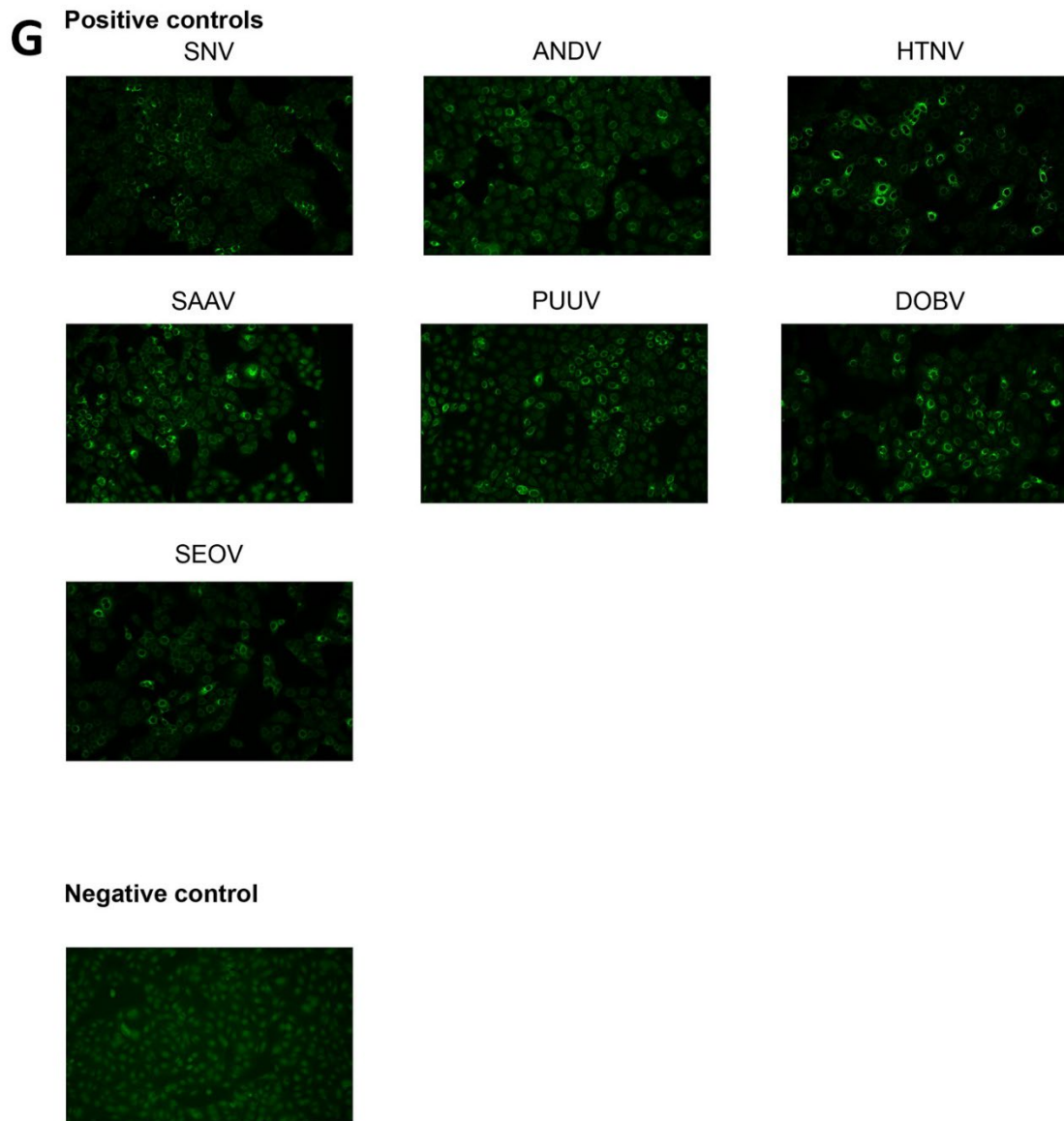


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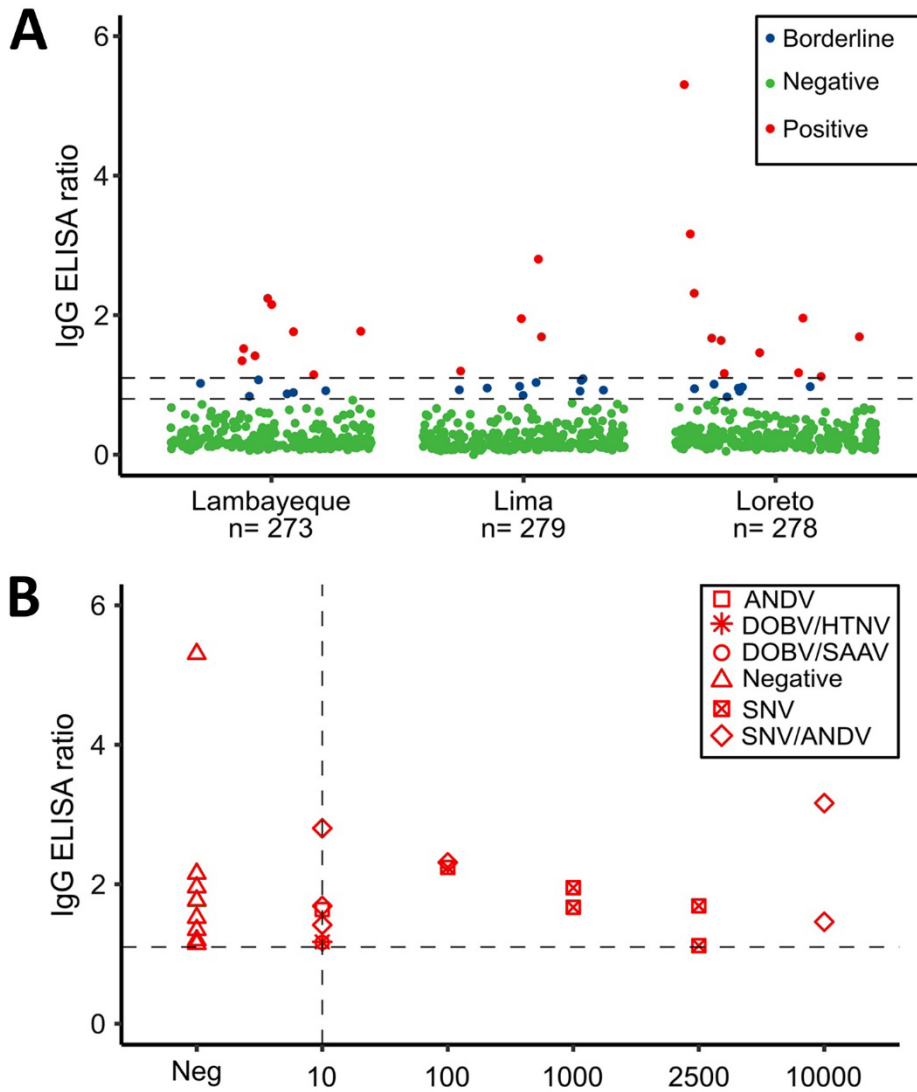


1:2500 ANDV





Appendix Figure 4. End-point titration using immunofluorescence assay (IFA) for IgM and IgG antibodies against Sin Nombre virus (SNV), Andes virus (ANDV), Seoul virus (SEOV), Hantaan (HTNV), Puumala (PUUV), Dobrava (DOBV), and Saaremaa (SAAV) hantaviruses. Samples IDs are indicated to the upper left of panels. All images were taken at 200 magnification.



Appendix Figure 5. Hantavirus seroepidemiology in Peru. A) ANDV and SNV IgG reactivities in 3 departments of Peru, between January 2020 and January 2021. B) ANDV- and SNV-positive serum samples in IgG ELISA compared to IgG IFA titers. DOBV, Dobrava virus, HTNV, Hantaan virus, SAAV, Saaremaa virus.