**Supplementary Material**

Supplement to: Holzbauer SM, Schrodt CA, et al. Fatal Human Rabies Infection with Suspected Host-mediated Failure of Post-Exposure Prophylaxis Following a Recognized Zoonotic Exposure— Minnesota, 2021.

**Table of contents**

1 Manufacturer lot numbers for human rabies immunoglobulin and vaccines 2

2 Case-patient clinical diagnostic results prior to rabies diagnosis 2

3 Manufacturer HRIG potency testing 5

4 Laboratory methods for bat species identification 5

5 Rabies diagnostics 6

5.1 Laboratory methods 6

5.2 Post-mortem histology 6

6 Laboratory methods for rabies virus sequencing 6

6.1 Sequencing 6

6.2 Sequencing analysis 7

6.3 Sequencing results 7

7 Influenza, SARS-CoV-2, and HCoV Serology 8

7.1 Laboratory methods 8

7.2 Results 8

8 Laboratory methods for HRIG potency testing 12

9 Online rabies healthcare assessment tool 12

10 Rabies exposure risk assessments in the U.S. 12

11 Supplemental References 12

# Manufacturer lot numbers for human rabies immunoglobulin and vaccines

**Supplementary Table 1.** Rabies post-exposure prophylaxis (PEP) biologics with lot numbers received by the patient and his wife

|  |  |  |
| --- | --- | --- |
| **Date** | **Patient** | **Patient’s wife** |
| 30JUL2020 | Total 1700IU HRIG (300 units/mL) in right hand around bite site with remaining administered into right thigh   * 1500 IU HRIG   Grifols HyperRab  Lot n° R2MFD00163 (Exp. 15Jun2021)   * 200 IU HRIG   Grifols HyperRab  Lot n° R2MBD00113† (Exp. 11May2021)  2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ARBA651B֍ | 1300IU of HRIG  Grifols HyperRab  Lot n° R2MFD00103, Exp. 01Apr2021)  2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ARBA651B֍ |
| 2AUG2020 | 2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ARBA651B֍ | 2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ADO1A20A |
| 6AUG2020 | 2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ARBA651B֍ | 2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ARBA571A |
| 13AUG2020 | 2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ARBA651A\* | 2·5IU of rabies vaccine IM RabAvert (GlaxoSmithKline)  Lot n° ARBA651A\* |

†Only PEP product remaining at hospital during investigation and tested.

֍\*: denotes common vaccine lot between patient and his wife.

HRIG: Human Rabies Immunoglobulin; IU: International Units.

# Case-patient clinical diagnostic results prior to rabies diagnosis

**Supplementary Table 2.** Clinical diagnostic test results prior to rabies diagnosis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Test type, specimen | Specimen collection date | Result | Units/Reference Range | Interpretation |
| Hemoglobin A1c | 25 August 2020 | 8.2 | 4.0-5.6% | High |
| Glucose, serum | 25 August 2020 | 150 | 70-99 mg/dL | High |
|  |  |  |  |  |
| Erythrocyte Sedimentation Rate | 9 January 2021 | 110 | 0–20 mm/hr | High |
| Glucose, serum | 9 January 2021 | 162 | 70-99 mg/dL | High |
|  |  |  |  |  |
| Lumbar Puncture 1, CSF | 15 January 2021 |  |  |  |
| Red Blood Cell Count |  | 0 | ≤0 cu mm | ·· |
| Protein |  | 66 | 15–45 mg/dL | High |
| Glucose |  | 152 | mg/dL | ·· |
| Total Nucleated Cell Count |  | 10 | 0–5 cu mm | High |
| Segmented Neutrophils |  | 4 | 0–6% | ·· |
| Lymphocytes |  | 72 | 40–80% | ·· |
| Mononuclear cells |  | 24 | 15–45% | ·· |
| Lumbar Puncture 2, CSF | 20 January 2021 |  |  |  |
| Red Blood Cell Count |  | 548 | ≤0 cu mm | High |
| Protein |  | 344 | 15–45 mg/dL | High |
| Glucose |  | 146 | mg/dL | ·· |
| Total Nucleated Cell Count |  | 28 | 0–5 cu mm | High |
| Segmented Neutrophils |  | 29 | 0–6% | High |
| Lymphocytes |  | 43 | 40–80% | ·· |
| Mononuclear cells |  | 28 | 15–45% | ·· |
|  |  |  |  |  |
| Complete Blood Count | 14 January 2021 |  |  |  |
| White Blood Cell Count |  | 12·5 | 3·2–11·0 10^9/L | High |
| Hemoglobin |  | 14·0 | 12·9–16·9 g/dL | ·· |
| Hematocrit |  | 41·3 | 38·4–49·7 % | ·· |
| MCV |  | 89·2 | 81·4–99·0 fL | ·· |
| Platelets |  | 241 | 130–375 10^9/L | ·· |
| Comprehensive Metabolic Panel | 14 January 2021 |  |  |  |
| Sodium |  | 132 | 134–143 mEq/L | Low |
| Potassium |  | 4·2 | 3·4–5·1 mEq/L | ·· |
| Chloride |  | 95 | 99–110 mEq/L | Low |
| Carbon dioxide (CO2) |  | 23 | 19–29 mEq/L | ·· |
| Blood Urea Nitrogen |  | 26 | 5–24 mg/dL | High |
| Creatinine |  | 1·14 | 0·70–1·20 mg/dL | ·· |
| Glucose |  | 331 | 70–99 mg/dL | High |
| Protein, Total |  | 8·1 | 6·0–8·0 g/dL | High |
| Calcium |  | 8·6 | 8·4–10·5 mg/dL | ·· |
| Albumin |  | 3·6 | 3·5–5·0 g/dL | ·· |
| Bilirubin, total |  | 2·3 | 0·2–1·2 mg/dL | High |
| Alkaline Phosphatase |  | 167 | 40–150 IU/L | High |
| Alanine Aminotransferase |  | 12 | 6–40 IU/L | ·· |
| Aspartate Aminotransferase |  | 13 | 10–40 IU/L | ·· |
|  |  |  |  |  |
| Myeloma/MGUS Screen with Immunoglobulins, Serum | 14 January 2021 |  |  |  |
| Protein, total |  | 6·8 | 6·0–8·0 g/dL | ·· |
| IgA |  | 52 | 85–370 mg/dL | Low |
| IgG |  | 485 | 661–1464 mg/dL | Low |
| IgM |  | 2699 | 40–275 mg/dL | High |
| Myeloma/MGUS M-Spike Surveillance, Serum | 14 January 2021 |  |  |  |
| Protein, Total |  | 6·8 | 6·0–8·0 g/dL | ·· |
| Albumin, ELP |  | 2·96 | 3·20–4·40 g/dL | Low |
| Alpha 1 |  | 0·22 | 0·20–0·40 g/dL | ·· |
| Alpha 2 |  | 0·78 | 0·50–1·10 g/dL | ·· |
| Beta 1 |  | 0·62 | 0·60–1·30 g/dL | ·· |
| Gamma |  | 2·23 | 0·70–1·60 g/dL | High |
| Gamma Monoclonal Protein (M-Spike) |  | 1·86 | g/dL | High |
| MYD88 L265P alteration | 14 January 2021 | Negative |  | ·· |
| Immunoglobulins\* | 21 January 2021 |  |  |  |
| IgA |  | 19 | 85–370 mg/dL | Low |
| IgG |  | 110 | 661–1464 mg/dL | Low |
| IgM |  | 470 | 40–275 mg/dL | High |
| Viscosity, serum | 19 January 2021 | 1·4 | 1·4–1·8 | ·· |
| Free Light Chains, serum | 19 January 2021 |  |  |  |
| Kappa |  | 1·07 | 0·33–1·94 mg/dL | ·· |
| Lambda |  | 1·77 | 0·57–2·63 mg/dL | ·· |
| Kappa/Lambda ratio |  | 0·6045 | 0·26–1·65 | ·· |
| Free Light Chains, CSF | 15 January 2021 |  |  |  |
| Kappa |  | 0·0152 | <0·1000 mg/dL | ·· |
|  |  |  |  |  |
| Autoimmune, serum | 14 January 2021 |  |  |  |
| Antinuclear Antibodies Screen |  | 0·17 | 0–0·99 Units | ·· |
| Hexosaminidase A (MUGS) |  | 1·90 | 1·23–2·59 U/L | ·· |
| Muscle–specific Kinase autoantibody |  | 0·00 | 0·00–0·02 mmol/L | ·· |
| *Antineutrophil cytoplasmic Antibodies Vasculitis Panel, Serum* | 14 January 2021 |  |  |  |
| Myeloperoxidase Antibodies IgG |  | <0·2 | <0·4 (Negative) Units | ·· |
| Proteinase 3 Antibodies IgG |  | <0·2 | <0·4 (Negative) Units | ·· |
| *Early Sjogrens Syndrome Antibody Profile, Serum* | 14 January 2021 |  |  |  |
| Salivary Protein 1 (SP-1) IgG |  | 13·4 | <20 EU/ml | ·· |
| Salivary Protein 1 (SP-1) IgA |  | 3·8 | <20 EU/ml | ·· |
| Salivary Protein 1 (SP-1) IgM |  | 14·0 | <20 EU/ml | ·· |
| Carbonic Anhydrase VI (CA VI) IgG |  | 6·8 | <20 EU/ml | ·· |
| Carbonic Anhydrase VI (CA VI) IgA |  | 6·8 | <20 EU/ml | ·· |
| Carbonic Anhydrase VI (CA VI) IgM |  | 12·6 | <20 EU/ml | ·· |
| Parotid Specific Protein (PSP) IgG |  | 8·0 | <20 EU/ml | ·· |
| Parotid Specific Protein (PSP) IgA |  | 2·5 | <20 EU/ml | ·· |
| Parotid Specific Protein (PSP) IgM |  | 22·6 | <20 EU/ml | Borderline |
| *Myasthenia Gravis Evaluation, Serum* | 14 January 2021 |  |  |  |
| Acetylcholine Receptor Binding Antibody |  | 0·00 | ≤0·02 nmol/L | ·· |
| ACh (Muscle) Modulating Antibody |  | 5 | 0–20% (reported as % loss of AChR) | ·· |
| Striated Muscle Ab |  | Negative | <1:120 titer | ·· |
| Paraneoplastic Autoantibody Evaluation, CSF | 15 January 2021 |  |  |  |
| Anti-Glial Nuclear Antibody |  | Negative | <1:2 titer | ·· |
| Amphiphysin Antibody |  | Negative | <1:2 titer | ·· |
| ANNA-1 |  | Negative | <1:2 titer | ·· |
| ANNA-2 |  | Negative | <1:2 titer | ·· |
| ANNA-3 |  | Negative | <1:2 titer | ·· |
| CRMP-5-IGG |  | Negative | <1:2 titer | ·· |
| PCA-1 |  | Negative | <1:2 titer | ·· |
| PCA-2 |  | Negative | <1:2 titer | ·· |
| PCA-TR |  | Negative | <1:2 titer | ·· |
|  |  |  |  |  |
| Infectious, serum |  |  |  |  |
| Bacterial culture | 15 January 2021 | No growth after 5 days incubation | No growth | ·· |
| *Treponema pallidum* Antibody | 14 January 2021 | Nonreactive | Nonreactive |  |
| HIV Antigen/Antibody | 14 January 2021 | Nonreactive | Nonreactive | ·· |
| *Borrelia burgdorferi* IgM Antibody | 14 January 2021 | Negative | Negative | ·· |
| *Borrelia burgdorferi* IgG Antibody | 14 January 2021 | Negative | Negative, Equivocal | ·· |
| Infectious, CSF | 15 January 2021 |  |  |  |
| Gram stain |  | No organisms detected | No organisms detected | ·· |
| Bacterial culture |  | No growth | No growth | ·· |
| Fungal culture |  | No growth | No growth | ·· |
| Herpes Simplex Virus 1 and 2 PCR |  | Negative | Negative | ·· |
| Varicella-Zoster PCR |  | Negative | Negative | ·· |
| *Borrelia burgdorferi* IgG Antibody |  | Negative | Negative | ·· |
| VDRL† |  | Negative | Negative | ·· |
| *Streptococcus pneumoniae* antigen |  | Negative | Negative | ·· |
| West Nile Virus IgM |  | Negative | Negative | ·· |
| West Nile Virus IgG |  | Negative | Negative | ·· |
| *Borrelia burgdorferi* PCR |  | Negative | Negative | ·· |
| *Borrelia mayonii* PCR |  | Negative | Negative | ·· |
| *Borrelia garinii/Borelia afzelii* PCR |  | Negative | Negative | ·· |
| Lymphocytic choriomeningitis virus PCR |  | Negative | Negative | ·· |
| Cytomegalovirus PCR |  | Negative | Negative | ·· |
| Miscellaneous |  |  |  |  |
| SARS-CoV-2 PCR, nasal swab | 9 January 2021 | Negative | Negative | ·· |
| SARS-CoV-2 PCR, NP swab | 14 January 2021 | Negative | Negative | ·· |
| Respiratory culture, sputum | 15 January 2021 | Normal respiratory flora |  | ·· |
| Gram stain, sputum | 15 January 2021 | Many mixed flora |  | ·· |

Abbreviations: LP: Lumbar Puncture; CSF: Cerebrospinal Fluid; MGUS: Monoclonal Gammopathy of Undetermined Significance; Ig: Immunoglobulin; NP: Nasopharyngeal; PCR: Polymerase Chain Reaction; HIV: Human Immunodeficiency Virus; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; VDRL: Venereal Disease Research Laboratory.

\*After plasmapheresis was performed on 16 January, 18 January, and 20 January.

†Nontreponemal screening test for syphilis.

# Manufacturer HRIG potency testing

**Supplementary Table 3.** Human rabies immunoglobulin manufacturer Rapid Fluorescent Foci Inhibition Test (RFFIT) potency test results

|  |  |
| --- | --- |
| **Grifols HyperRab Lot Numbers** | **RFFIT Potency Test Results** |
| Lot n°R2MFD00163  (Exp. 15Jun2021)  Lot n° R2MBD00113  (Exp. 11May2021) | 440 IU/kg    421 IU/kg |

# Laboratory methods for bat species identification

Total RNA was extracted from rabies positive brain tissues using RNeasy Mini Kit (Cat # 75136, Qiagen, Germantown, MD, USA) utilizing a modified protocol as previously described.[1, 2] Specifically, approximate 340 bp long 12S rRNA fragment was amplified with 2·5uL RNA extract, 22·5uL RT-PCR master mix containing 1 x buffer, 400µM dNTP, 1µL enzyme mix from Qiagen One-step RT-PCR Kit (Cat. 210212, Qiagen), 400µM forward primer Bat-1: 5’-TAA AGG AGC TGG TAT CAA GC-3’, and reverse primer Bat-2: 5’-GGG TAT CTA ATC CCA GTT TG-3’. Thermal cycling conditions are 50°C for 30mn for RT, followed by 95°C for 15mn, then 40 cycles of 95°C, 15s, 50°C, 30s, 72°C, 45s. Purified RT-PCR product with QIAquick PCR Purification Kit (Cat. #28104 QIAGEN was sequenced with GenomeLab Dye Terminator Cycle Sequencing with Quick Start Kit and Beckman Coulter CEQ 8000 Genetic Analysis System (17909, Beckman Coulter, East Lyme, CT, US) following manufacturer’s instructions.

SCF sequence files off sequencer were analyzed and assembled with software Geneious Primer® (2019.2.1) ([https://www.geneious.com](https://www.geneious.com/)). Resulting approximate 300 bp long sequence was queried against Genbank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch>). The query sequence was found 100% identical to reference *Lasionycteris* *noctivagans* (Silver-haired bat, SHB) 12S ribosomal RNA gene (AF326095) sequence.

# Rabies diagnostics

## Laboratory methods

Antemortem nuchal skin biopsy and postmortem CNS tissues (brain stem, vermis, right cerebellum, left cerebellum) were subjected to direct fluorescent antibody (DFA) test for rabies virus antigen detection as previously described using fluorescein-isothiocynate (FITC) conjugated anti-RABV monoclonal antibodies.[3, 4] These samples, along with antemortem saliva, were tested for the presence of RABV nucleic acid by the LN34 pan-lyssavirus Taqman real time RT-PCR assay.[5, 6] Rabies serology was performed on serum and cerebrospinal fluid (CSF) for detection of anti-rabies antibodies. Rabies virus neutralizing antibodies (RVNA) were detected using rapid fluorescent focus inhibition test (RFFIT) using the laboratory standard CVS-11 RABV variant[7], while binding antibodies including differentiation of IgM and IgG antibody isotype specific responses were detected by indirect fluorescence antibody (IFA) test using CVS-11 infected cells.[8] Immunohistochemistry was performed on autopsy brain tissue using an indirect immuno-alkaline phosphatase detection system. Four micron tissue sections were deparaffinized, rehydrated, and underwent heat-induced epitope retrieval with citrate, prior to application of rabbit hyperimmune rabies virus antiserum.[9] After sequential application of biotinylated linked antibody, avidin-alkaline phosphatase complex, and naphthol fast-red substrate, sections were counterstained in Meyer’s hematoxylin and mounted with aqueous mounting medium.

## Post-mortem histology

Hematoxylin and Eosin (H&E) staining of vertebral bone marrow (Figure 2A) revealed prostatic adenocarcinoma (Grade Group 5, Gleason grade 5 + 5 = 10) with no evidence of a hematologic malignancy. Multiple brain sections showed evidence of rhabdoviral meningoencephalitis with immunohistochemical evidence of rabies virus. Examination of H&E stained medulla (Figure 2B) showed widespread neuronal viral cytopathic effect with variably ovoid to elongate sharply delineated eosinophilic cytoplasmic inclusions (arrows) approximately ~ 10-25 microns in size (Negri bodies). Cerebellum (Figure 2C) and brain stem (Figure 2D) showed extensive labeling of rabies viral antigen by immunohistochemistry (IHC) within the cytoplasm of neurons, and to a lesser extent within the neuropil. Note that the globular appearance of IHC staining correlates with the appearance of Negri bodies in H&E stained sections.

# Laboratory methods for rabies virus sequencing

## Sequencing

Total RNA was extracted from rabies positive brain samples using the Direct-zol RNA miniprep kit (R2051 Zymo, Irvine, CA, USA) as previously described.[5] cDNA was using primer 1F (**Supplementary Table 3**), Protector RNase Inhibitor (3335399001 Roche, Sigma-Aldrich, St. Louis, MO, USA), and Roche AMV reverse transcriptase (10109118001 Roche) at 42 °C for 90mn followed by heat inactivation for 10mn at 95°C. cDNA was then diluted by adding 75µL nuclease-free water to each sample. Whole genome amplification was performed using overlapping primer sets designed against U.S. *Lasionycteris noctivagans* rabies virus variant using reference JQ685895 (**Supplementary Table 3**) using Takara long amplicon Taq with GC buffers (RR02AG Takara Bio USA, Mountain View, CA, USA). PCR products were pooled for each sample, cleaned-up using MinElute PCR Purification kit (Qiagen), eluted in 40µl, and diluted to a volume of 130µl. DNA was sheared using a Covaris S220 instrument to approximately 650 bp. Library preparation was performed using Swift Accel-NGS 2S Plus DNA Library Kit with 2S Set A+B Indexing Kit according to the manufacturer’s instructions. Libraries were checked using an Agilent 2200 Tapestation prior to sequencing. Sequencing was performed on an Illumina MiSeq using MiSeq Reagent Kit v3 (600-cycle) (Illumina MS-102-3003). Sample A21-1091 required additional sequencing because few reads were produced in the first sequencing run. RNA was re-extracted from rabies positive brain samples using the Direct-zol RNA miniprep kit (R2051 Zymo, Irvine, CA, USA) including the on the column DNase I treatment, following the manufacturer’s protocol. First strand cDNA was generated following the above protocol using LN34 forward primers.[5] DNA was sheared using a Covaris S220 instrument to approximately 400 bp. Library preparation was performed using Swift Accel-NGS 1S Plus DNA Library Kit with 1S Plus Combinatorial Dual Indexing Kit (12 x 8) according to the manufacturer’s instructions. The library was checked using an Agilent 2200 Tapestation, and sequencing was performed using MiSeq Reagent Kit v3 (600-cycle) (Illumina MS-102-3003).

**Supplementary Table 4.** Primers used for rabies virus whole genome sequencing

|  |  |
| --- | --- |
| Name | Sequence |
| 1F | ACGCUUAACGACAAAAUCAGAG |
| 500F | CCTAAGTTTGTATAGGTTGAGC |
| 1800R | CCTGACTTCATCTGTCTGAC |
| 2500F | AAAAACAGGCAACACCACTG |
| 2500R | TCATCTCTGCAGGTCTTCACT |
| 4400F | ATGGAAGCTGATGCTCATTAC |
| 4400R | CAACCTTTTGAGGGGATGAC |
| 6000F | UAUGGGGAUUGCUGAUUGUGAC |
| 6500R | TGCCCCCAATGTCTGTAAC |
| 7400F | UAUUCAGACAGAUCUGACCUC |
| 7800R | GCTACACATGGTCTCCTC |
| 9100F | AACATAATGTCTCTGACAGGCCC |
| 9300R | GTCCATGTCTGTGCATAAAGC |
| 10400F | TGGGTCTGCTCTGCTCAACA |
| 11100R | CCTCTGCATCTCGCTCTTGG |
| 11900R | ACGCTTAACAAAAAAACAATAAAGA |

## Sequencing analysis

Analysis was performed in CLC Genomics Workbench v20.0.3. Reads were trimmed using default settings with additional trimming of homopolymers and 10 bp from each end to remove primer sequences. Trimmed reads were mapped to reference JQ685895 using standard settings. A draft genome was extracted from the mapped reads using default settings. Sequences were deposited to Genbank under accession numbers TBD. Genomes were aligned using Mafft v.7.450 in Geneious Prime 2019.1.1 Phylogenetic analysis was performed in BEAST 1.10.4 using GTR+G+I nucleotide substitution model, strict clock and constant coalescent population prior. Tree was rooted with reference JQ685919 (*Lasiurus borealis* rabies virus variant); JQ685922 and AY705373 are shown as outgroups in the tree. Maximum clade credibility tree was generated using TreeAnnotator (BEAST 2.6.3). Tree was annotated and colored in FigTree 1.4.4 and Inkscape ([inkscape.org](http://inkscape.org/)).

## Sequencing results

When compared with whole genome rabies virus variant sequences from five silver-haired bats collected in 2019 and 2020 from surrounding counties, between 31 and 102 nucleotide differences were observed, further supporting epidemiologic findings that the offending bat was the source of infection. Sequences of RABV isolates from silver-haired bats from Colorado and Washington were 43–185 nucleotides different (**Supplementary Table 4**). Posterior support values are shown at the branch points; scale bar is in substitutions per site.

**Supplementary Table 5.** Number of nucleotide differences between rabies virus whole genome sequences from human case and rabies virus from selected bat samples with *Lasionycteris noctivagans* rabies virus variant.

|  |  |  |
| --- | --- | --- |
| **Sample\*** | **Nucleotide differences** | **County, state, and year** |
| A21-1094 | 0 | MN 2020 |
| A21-1096 | 31 | Saint Louis County, MN 2020 |
| A19-3014 | 43 | La Plata County, CO 2019 |
| A18-3836 | 63 | Mesa County, CO 2018 |
| A21-1093 | 71 | Itasca County, MN 2020 |
| A21-1095 | 78 | Ramsey County, MN 2020 |
| JQ685895 | 82 | WA 2003 |
| A21-1091 | 88 | Olmsted County, MN 2019 |
| A21-1097 | 102 | Anoka County, MN 2020 |
| A18-3837 | 185 | Boulder County, CO 2018 |

\* Sample ID corresponds to those shown in Figure 3.

# Influenza, SARS-CoV-2, and HCoV Serology

## Laboratory methods

Serum antibodies to influenza were detected by hemagglutination inhibition assays and a high-throughput multiplex Influenza/SARS-CoV-2/seasonal human coronavirus (HCoV) antibody detection assay. Hemagglutination inhibition assay was performed using method as previously described.[10] Briefly, nonspecific inhibitors in the sera were removed by incubation with receptor-destroying enzyme at 37°C for 18 to 20h, followed by heat inactivation at 56°C for 30mn. Serially 2-fold-diluted sera were tested in duplicate using 0·5% turkey erythrocytes. HI titer was defined as the reciprocal of the last dilution of serum that completely inhibited hemagglutination. Antibody titers less than 10 (initial sera dilution) were reported as 5 for calculation purposes. Sera samples were tested in duplicates, geometric mean titers of the duplicate HI titers were reported as the final titers.

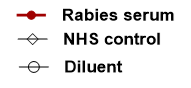
The high-throughput Influenza/SARS-CoV-2/HCoV virus antibody detection assay[11, 12] was used to detect the pan immunoglobulin (Ig), IgG, IgM, and IgA in the serum samples to a panel of influenza, SARS-CoV-2, and human coronavirus antigens. The influenza antigens were either obtained from the International Reagent Resource (IRR) (<https://www.internationalreagentresource.org/About/IRR.aspx>) or expressed and purified using an in-house baculovirus expression system. Coronavirus antigens were obtained from Sino Biological US Inc. (PA, USA). Antigens were conjugated to Bio-Plex Pro magnetic COOH beads as previously described.[11] Briefly, fifty microliters of microspheres containing 2,000 microspheres for each conjugated antigen were added to each well of a black-wall plate, followed by the incubation of 1:500-diluted human serum samples in assay buffer (PBS with 0·5M NaCl, 0·05% Tween-20, 1% BSA, and 0·05% sodium azide) at room temperature for one hour. After washes with assay buffer, PE (R-phycoerythrin) conjugated goat F(ab')2 Anti-Human total Ig reporter (reacts with the heavy and light chain of IgG, IgM and IgA), or PE-conjugated goat F(ab')2 anti-human IgG, or PE conjugated goat F(ab')2 anti-human IgM, or PE-conjugated goat F(ab')2 antihuman IgA (Southern Biotech, Inc. AL) in assay buffer were added and incubated at room temperature for one hour, followed by 3 washes with reading buffer (PBS with 0·05% Tween-20, 1% BSA, and 0·05% sodium azide). The median fluorescence intensity (MFI) was obtained by a Bio-Plex® MAGPIX™ Multiplex Reader. The samples were tested in duplicate, the mean of the duplicate was reported as the final MFI.

## Results

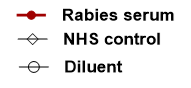
The patient received high dose quadrivalent influenza vaccine on August 25, 2020. A single serum sample collected on January 15, 2021, 143 days post influenza vaccination (172 days post the bat bite, 9 days pre-mortem) was analyzed for the presence to the antibodies to influenza. The patient was seropositive by hemagglutination inhibition assays (HI≥40) to 3 of the 4 vaccine components, including A(H3N2), B/Victoria and B/Yamagata, seronegative for A(H1N1)pdm09 (**Supplementary Table 6**). The serum sample was also analyzed for pan immunoglobulin (Ig), IgG, IgM and IgA antibodies to a panel of influenza, SARS-CoV-2, and seasonal human corona virus antigens by a high throughput multiplex antibody detection assay (**Supplementary Figure 1**). High Pan immunoglobulin (Ig) and IgG MFI titers to a panel of hemagglutinins (HAs) from several recent seasonal influenza A(H1N1), A(H3N2), B/Victoria and B/Yamagata viruses as well as to the influenza nucleoprotein (**Supplementary Figure 1 A and B**) were detected. Moderate levels of IgM MFI titers were also detected to HAs from some A(H1N1), A(H3N2) and B viruses, with very low or no IgA MFI titers to any antigens tested in the panel (**Supplementary Figure 1 C and D**). No antibodies to ectodomain, receptor binding domain (RBD), S1 and S2 of the spike protein of SARS-CoV-2 virus, and no antibodies to SARS-CoV-2 nucleoprotein (N) were found, indicating the patient had no SARS-CoV-2 infection. Low levels of Pan Ig and IgG MFI titers to seasonal human coronavirus OC43 were detected indicating possible past exposure.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Supplementary Table 6.** Hemagglutination inhibition titers to influenza vaccine antigens in a single serum collected from the rabies patient premortem | | | | |
| **Subtype** | **Viruses** | **Passage** | **HI titer** | **Seropositivity\*** |
| A(H1N1)pdm09 | A/Victoria/2454/2019 | egg | 5 | seronegative |
| A(H1N1)pdm09 | A/Hawaii/70/2019 | cell | 10 | seronegative |
| A(H3N2) | A/Hong Kong/2671/2019 | egg | 160 | seropositive |
| B/Victoria | B/Washington/02/2019 | egg | 160 | seropositive |
| B/Yamagata | B/Phuket/3073/2013 | egg | 80 | seropositive |

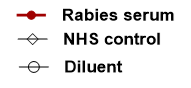
\*Seropositive is defined as HI titer ≥ 40.



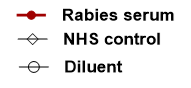
**Supplementary Figure 1, A.** Pan Immunoglobulin to Influenza, SARS-CoV-2, and human coronaviruses in premortem serum by multiplex assay



**Supplementary Figure 1, B.** IgG antibodies to Influenza, SARS-CoV-2, and human coronaviruses in premortem serum by multiplex assay



**Supplementary Figure 1, C.** IgM antibodies to Influenza, SARS-CoV-2, and human coronaviruses in premortem serum by multiplex assay



**Supplementary Figure 1, D.** IgA antibodies to Influenza, SARS-CoV-2, and human coronaviruses in premortem serum by multiplex assay

# Laboratory methods for HRIG potency testing

We evaluated HRIG potency using RFFIT against CVS-11 variant and the silver-haired bat isolate using methods previously described [7] with some modification. Briefly, neutralization titer was determined at neat and various starting dilutions (1:2.5; 1:12.5; 1:62.5; 1:312.5 and 1:1562.5) of HRIG and based on the titers at each dilutions mean titer was calculated in reference to standard RIG (SRIG, 2 IU/ml). HRIG titer was also calculated by RFFIT at different dilutions and mean titer determined against the variant isolated from the patient.

# Online rabies healthcare assessment tool

The online rabies assessment of healthcare personnel was modeled after a previously published tool (available online at <https://www.cdc.gov/mmwr/volumes/69/wr/mm6929a3.htm?s_cid=mm6929a3_w>  
and <https://stacks.cdc.gov/view/cdc/90520>) used in 2019 by Whitehouse et al. in Utah [13]. Study data were collected and managed using REDCap electronic data capture tools hosted at Minnesota Department of Health [14, 15]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

[2021 Minnesota Human Rabies Healthcare Risk Assessment Tool (pdf)](https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fstacks.cdc.gov%2Fview%2Fcdc%2F116165&data=04%7C01%7Cstacy.holzbauer%40state.mn.us%7Ca7322410ff88421208bc08da1d49a366%7Ceb14b04624c445198f26b89c2159828c%7C0%7C0%7C637854499365286353%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000&sdata=%2Bq1jlcQoe%2BmFy7%2BZPdaZCrRojNe1UApLtnL0htGgngs%3D&reserved=0)

[2021 Minnesota Human Rabies Risk Healthcare Risk Assessment Tool REDCap Data Dictionary](https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fstacks.cdc.gov%2Fview%2Fcdc%2F116164&data=04%7C01%7Cstacy.holzbauer%40state.mn.us%7Ca7322410ff88421208bc08da1d49a366%7Ceb14b04624c445198f26b89c2159828c%7C0%7C0%7C637854499365286353%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000&sdata=ce2yLC9flPPypEH9S3sY7rK02wKZ9v%2BaW%2BAkhOHwYXs%3D&reserved=0)

# Rabies exposure risk assessments in the U.S.

Of 11 human rabies exposure risk assessments performed in the U.S. since 2011, an average of 197 persons (range: 71–297) were identified as close contacts, with PEP recommended for an average of 13.7% (range: 2.8–37.3%) of persons determined to be exposed [16-26].

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