Covid19-UFRJ Workgroup

The COVID19-UFRJ Workgroup encompasses all individuals (physicians, professors, technicians, postgraduate and undergraduate students, and volunteers) who participated in the COVID-19 diagnostics at the Universidade Federal do Rio de Janeiro during the COVID-19 pandemic.

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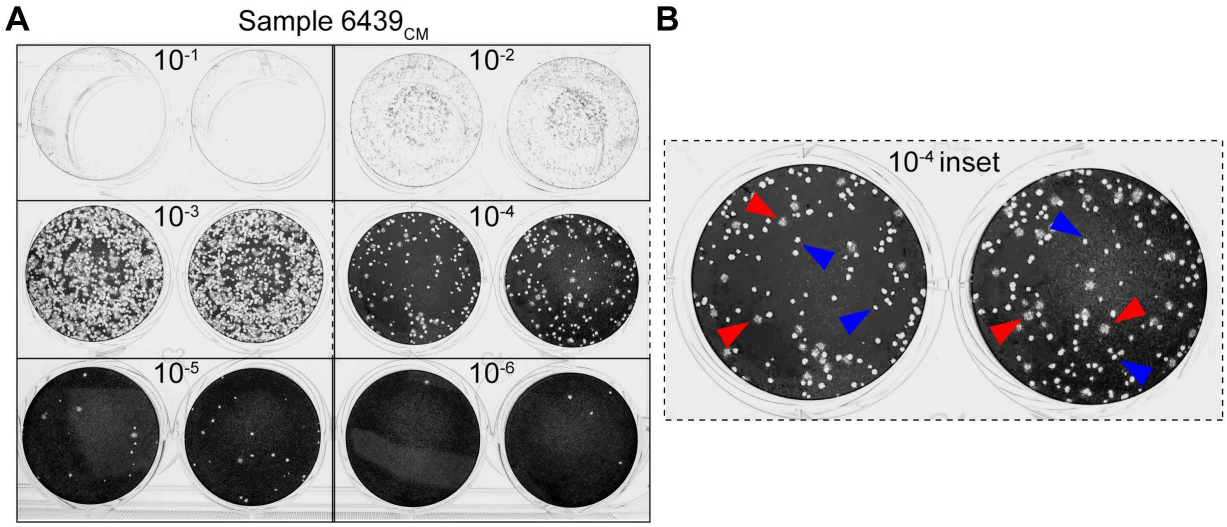

Fig. 1: plaque assay plate of the sample 6439_{CM} (A), showing the indicated inoculum dilutions in duplicate wells of infected Vero cell monolayers. The two plaque phenotypes observed in this sample are highlighted in the inset (B) of the 10^{-4} dilution duplicates (dashed panel). Red arrowheads correspond to the larger and turbid plaques with undefined borders, whereas blue arrowheads indicate the smaller and clearer plaques with well-defined circular borders.

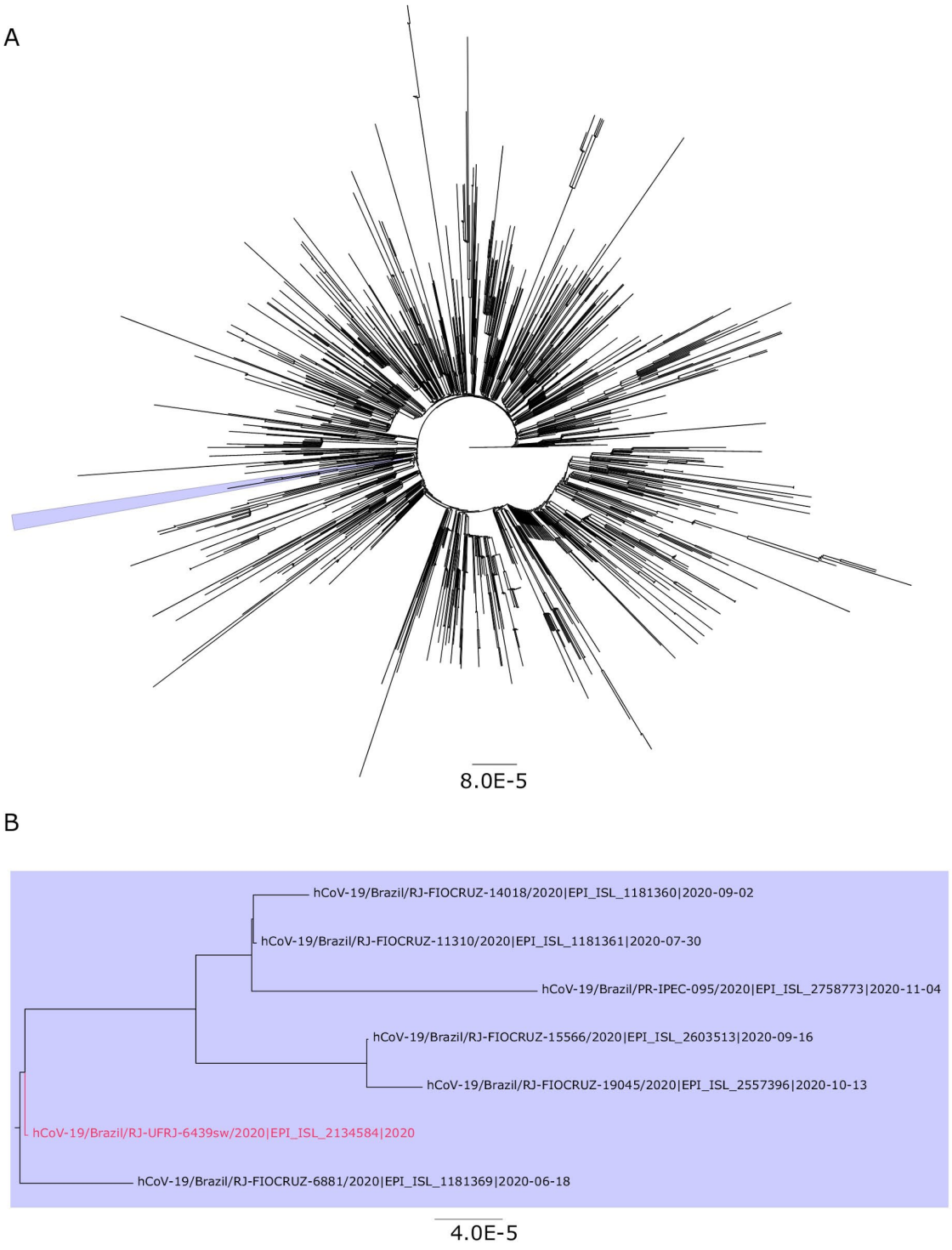

Fig. 2: phylogenetic characterisation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) obtained from the sample 6439_{sw}. (A) Maximum likelihood phylogenetic tree inferred from a dataset comprehending all Brazilian SARS-CoV-2 complete genome sequences from lineage B.1.1.33 available on GISAID EpiCoV database (as of July 17, 2021) (n = 1,307). The light purple shade highlights the clade where the novel genome clustered. (B) Zoom on the previous clade, revealing that the new characterised genome clusters as sister to a group comprehending four sequences from Rio de Janeiro and one from Paraná, though with low support value (SH-aLRT = 0) to the overall tree due to limited sequences divergence. The tree was arbitrarily rooted on the oldest sequence available in the dataset for visualisation purposes only. The scale bar indicates average substitutions per site.

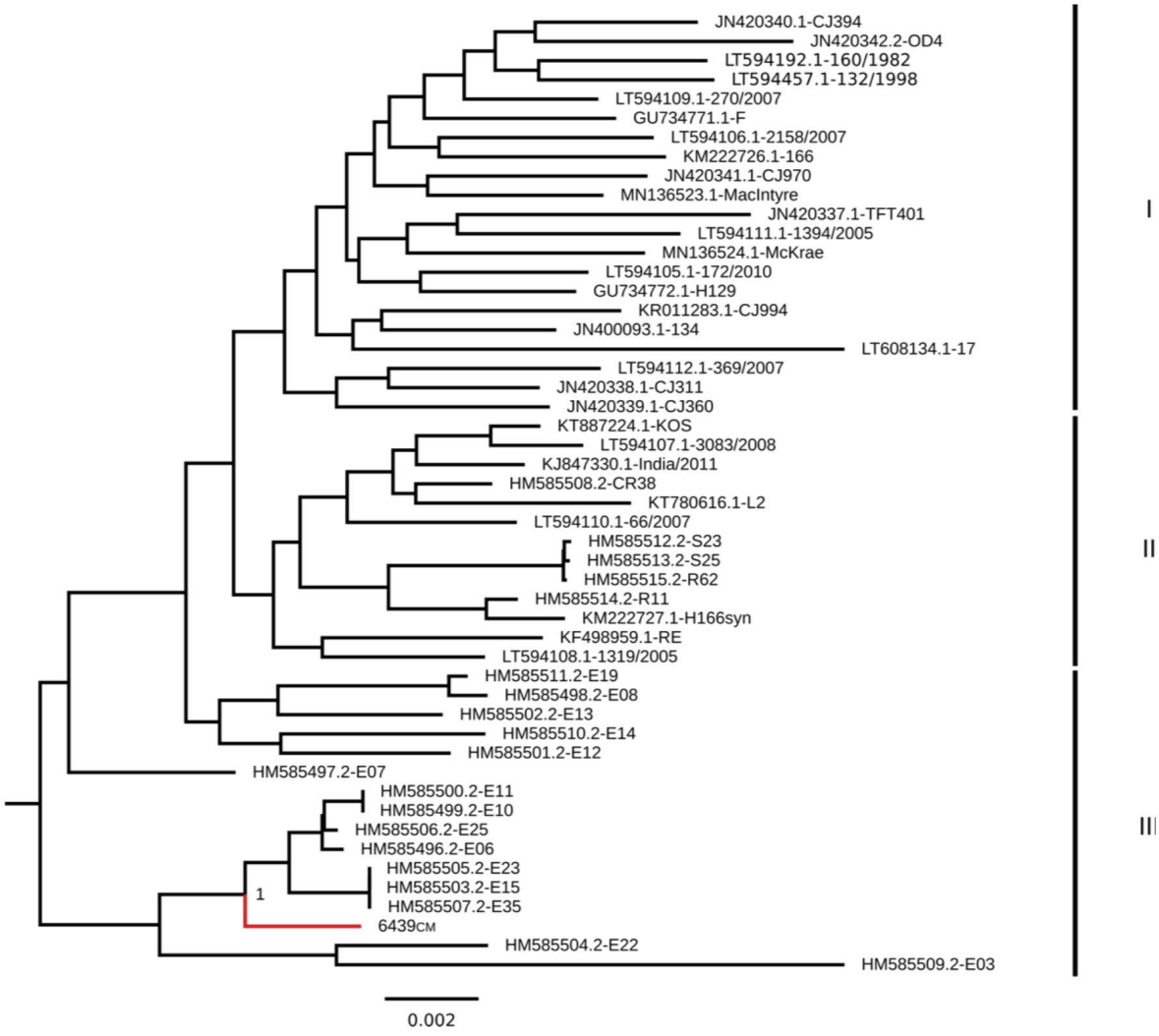

Fig. 3: maximum likelihood phylogeny containing the currently characterised diversity of alphaherpesviruses. The tree indicates that the HSV-1 strain herein described (6439_{CM}) clusters with maximum support as a sister to a clade composed of strains E11, E10, E25, E6, E23, E15, and E35. Black bars mark *Alphaherpesvirus* groups described in Pffaf et al.,[1] and the red branch indicates the HSV-1 sequence obtained from sample 6439_{CM}. The tree was midpoint rooted for visualisation purposes only. The scale bar indicates nucleotide substitutions per site.

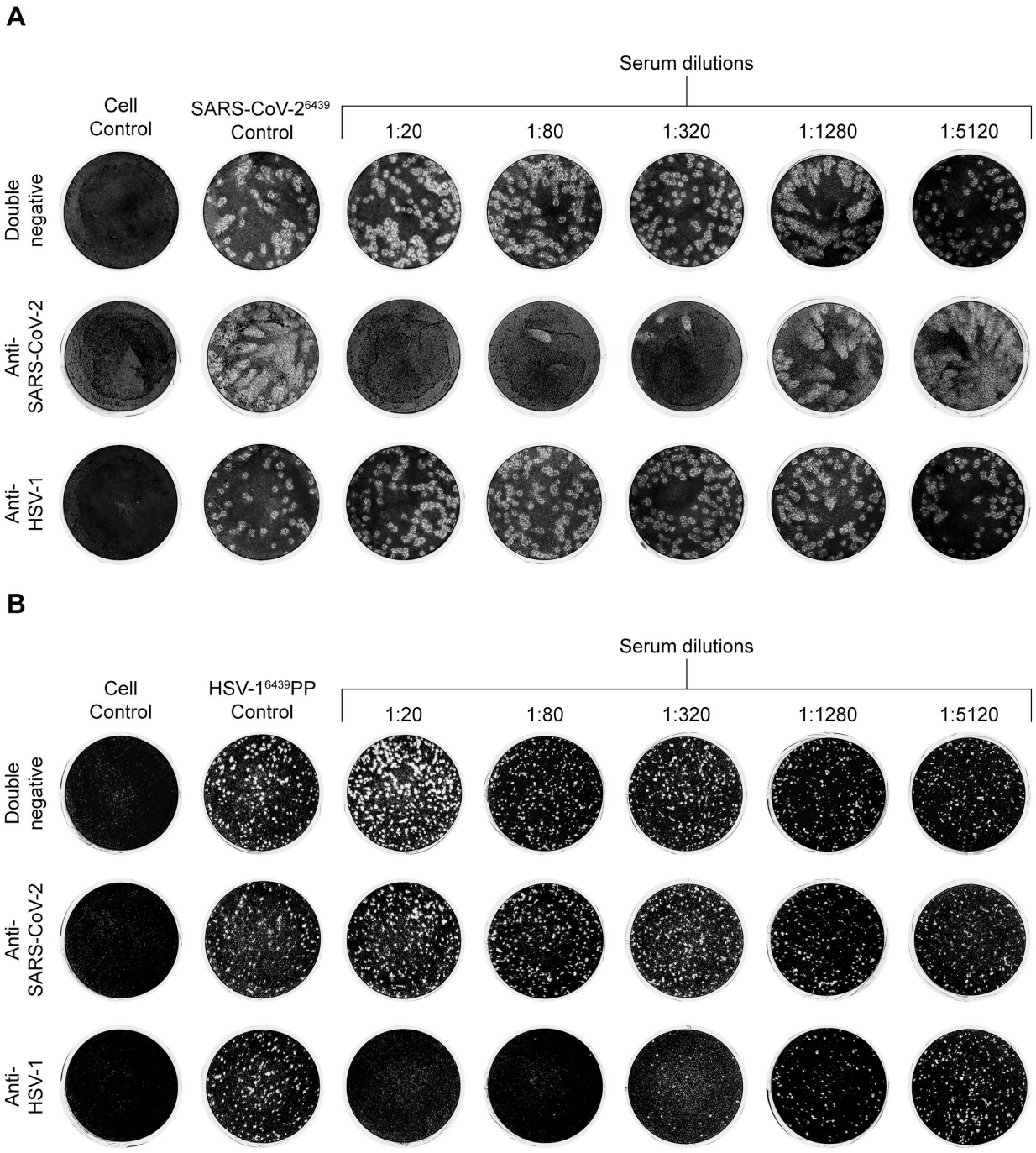

Fig. 4: plaque reduction neutralisation test (PRNT) with SARS-CoV-2^{6439} (A) and HSV-1^{6439} PP (B), the isolated viruses from 6439 samples using double-negative, anti-SARS-CoV-2, and anti-HSV sera. Columns correspond to fixed Vero cell monolayers subjected to the following conditions from left to right: uninfected cells (cell control), virus inoculated cells without serum (virus control), and cells inoculated with 100 PFU of virus pre-incubated with the indicated dilutions of the corresponding serum.

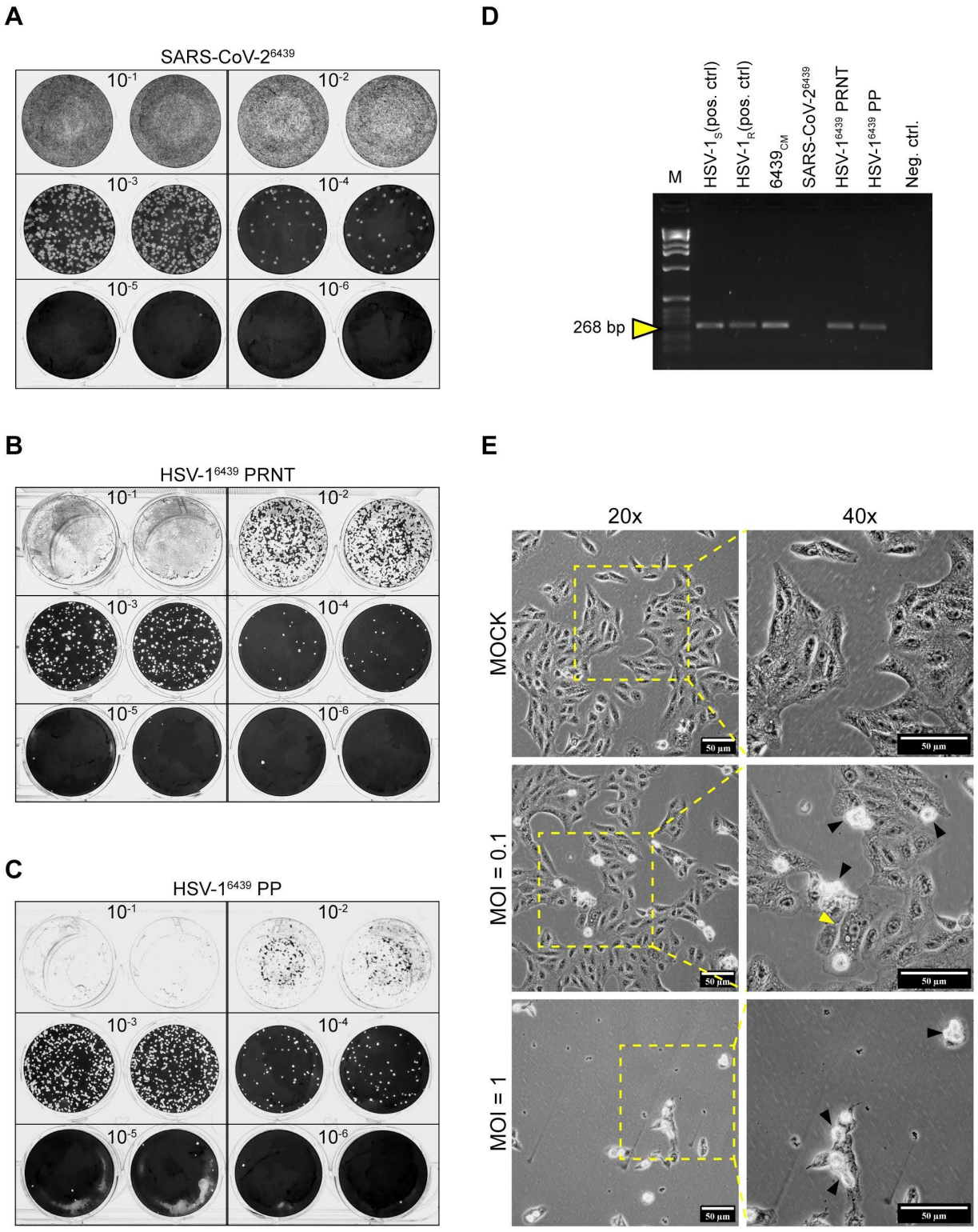

Fig. 5: plaque assay plates of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)^{6439}(A), Herpes simplex virus 1 (HSV-1)^{6439} plaque reduction neutralisation test (PRNT) (B), HSV-1^{6439} PP (C). Plates showing the indicated inoculum dilutions in duplicate wells of infected Vero cell monolayers. (D) HSV-1 detection was performed by polymerase chain reaction (PCR). Amplicons (268 bp) were visualised in 1.5% agarose gel electrophoresis. Lane M: 1 kb Plus DNA Ladder (Invitrogen); lanes 2 and 3: HSV-1 positive controls; lane 8: no template control. (E) Microscopy of Vero cells infected with purified HSV-1 from the sample 6439$_{CM}$ at MOI of 0.1 and 1 after 24 hpi. Panels on the right are magnifications of the correspondent yellow dashed inset. Black arrowheads indicate enlarged and round dying cells, and yellow arrowheads indicate cytoplasmic vacuolisation. White scale bars = 50 µm.

TABLE

The clinical course of patient 6439

Day	Date	Events	Symptoms/Clinical signs	Laboratory findings	Treatments
1	April 19	Presented at emergency care unit (ECU) where hospitalisation was requested for decompensated insulin-dependent diabetes mellitus	Decreased level of consciousness.	Ht-37.4%; WBC-10,500/mL (segmented neutrophils-45%, band forms-3%, lymphocytes-41%); blood urea-146mg/dL; creatinine-3.79mg/dl; elevated blood glucose	IV hydration, insulin
2	April 20	ECU	Decreased level of consciousness, Glasgow 9, eupneic in ambient air, dehydrated, physical examination without other significant changes.	Ht-41.4%, WBC-20,900/ml (band forms-8%)	eftriaxone, clindamycin, insulin
3	April 21	ECU, chest X-ray	Disoriented, eupneic in ambient air, reentrant seizures	Chest X-ray: right perihilar pulmonary infiltrate	Meropenem, vancomycin, hydantal, insulin
4	April 22	ECU	Aphasic, unresponsive, afebrile, pulmonary rhonchi and rales; SpO2:92% in ambient air;	Ht-41.4%, WBC-23,200/mL (band forms-10%); urea-176mg/dl; creatinine-5,2mg/dl; HGT-141-489mg/dl	Meropenem, vancomycin, hydantal, insulin
5	April 23	ECU	Not reported	Ht-41.4%, WBC-22,700/mL (band forms-10%); blood glucose-155mg/dl	Meropenem, vancomycin, hydantal, insulin
6	April 24	ECU, 1st Head CT	Head CT	WBC-13,700/mL (band forms-8%); urea- 142mg/dl; creatinine-4,5mg/dl. Head CT without alterations	Non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin
7	April 25	ECU	Disoriented, seizures, afebrile, pneumonia, uncontrolled hypertension, no other major changes	Not reported	Non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin, Nipride®
8	April 26	ECU	Disoriented but responsive, eupneic under nasal oxygen catheter, afebrile.	Ht-34,2%, WBC-17,300/mL (band forms-10%); urea- 214mg/dl, creatinine-6,2mg/dl; blood glucose-312mg/dl	Non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin, Nipride®
9	April 27	2nd Head CT and transfer to HFSE intensive care unit (ICU) for cerebrovascular accident (CVA) and pneumonia	Afebrile at admission, respiratory distress, Glasgow 13, left hemiparesis, dysarthria, sacral pressure ulcer, worsening renal function, decompensated diabetes.	No alterations in head CT.	Zero diet, nasogastric catheter, non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin, losartan, amlodipine, simvastatin, clexane
10	April 28	ICU, Hemodialysis started	No major evolutive changes	Ht-30,4%; WBC-13,700/mL; urea-217mg/dl, creatinine-5,7mg/dl, blood glucose-234mg/d; serum C-reactive protein-10.2mg/dl	Enteral diet, non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin, losartan, amlodipine, simvastatin, clexane, intermittent daily hemodialysis
11	April 29	ICU	Febrile (38ºC)	Ht-27%; WBC-10,100/mL; urea-110mg/dl; creatinine-3,0mg/dl; blood glucose-249mg/d; serum C-reactive protein-12.9mg/dl.	Enteral diet, non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin, losartan, amlodipine, simvastatin, clexane, intermittent daily hemodialysis
12	April 30	ICU, nasopharyngeal swab, head and thorax CT, notification to epidemiological surveillance	Disoriented, febrile (37.2-38.6ºC)	Ht-29.2%; WBC-14,300/mL; urea- 86/dl, creatinine-3,0mg/dl, blood glucose-126mg/dl, serum C-reactive protein-20.1mg/dl. Thorax CT with typical COVID-19 infiltrate. No alterations in head CT.	Enteral diet, non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin, losartan, amlodipine, simvastatin, clexane, ivermectin, intermittent daily hemodialysis
13	May 1	ICU	37.5°C; no major evolutive changes.	Ht-30% WBC-16,300/mL; urea-113/dl, creatinine-3,8mg/dl, blood glucose(plasma)-172mg/dl, serum C-reactive protein-20.1mg/dl	Enteral diet, non-invasive oxygen support, meropenem, vancomycin, hydantal, insulin, losartan, amlodipine, simvastatin, clexane, ivermectin, intermittent daily hemodialysis
14	May 2	ICU, Orotracheal intubation, and mechanical ventilatory support	Desaturation, arterial hypotension, torpor, afebrile	HGT:150-253mg?dL; serum C-reactive protein-21.6mg/dl	Invasive mechanical ventilation, enteral diet, meropenem, vancomycin, hydantal, simvastatin, clexane, insulin, noradrenaline, intermittent daily hemodialysis
15	May 3	ICU, Mechanical ventilatory support	Febrile-38.3°C, hemodynamic instability		Invasive mechanical ventilation, enteral diet, meropenem, vancomycin, hydantal, simvastatin, clexane, insulin, noradrenaline, intermittent daily hemodialysis
16	May 4	Death	Refractory shock and death at 6:15 am. Death declaration: Part I. Severe acute respiratory syndrome, COVID-19 pneumonia. Part II CVA - bronchoaspiration		

]ECU: emergency care unit; ICU: intensive care unit; CT: computed tomography; HSFE: Hospital Federal dos Servidores do Estado do Rio de Janeiro; CVA: cerebrovascular accident; Ht: blood hematocrit; WBC: white blood count; HGT: hemoglucotest; IV: intravenous.

REFERENCE

1. Pfaff F, Groth M, Sauerbrei A, Zell R. Genotyping of herpes simplex virus type 1 by whole-genome sequencing. J Gen Virol. 2016; 97(10): 2732-41.