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SARS-CoV-2 variants inactivation of plasma units using a riboflavin and ultraviolet light-based photochemical treatment

Check for updates

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ARTICLE INFO	A B S T R A C T
Keywords: Pathogen reduction SARS-CoV-2 Variants of concern Convalescent plasma donor Neutralizing antibodies Plasma transfusion therapy	<i>Background:</i> Test the ability of Mirasol Pathogen Reduction Technology (PRT, Terumo BCT, Lakewood Co, USA) treatment with riboflavin and ultraviolet light (R + UV) in reducing SARS-CoV-2 infectivity while maintaining blood product quality. <i>Material and methods:</i> SARS-CoV-2 strains were isolated and titrated to prepare cell free virus for plasma units infection. The units were then under treatment with Mirasol PRT. The infectious titers were determined before and after treatment with an in house microtitration assay on Vero E6 cells. Thirty-six plasma pool bags underwent PRT treatment. <i>Results:</i> In all the experiments, the measured titer following riboflavin and UV treatment was below the limit of detection of microtitration assay for all the different SARS-CoV-2 strains. Despite the high copies number detected by RT-PCR for each viral strain after treatment, viruses were completely inactivated and not able to infect VERO E6 cells. <i>Conclusion:</i> Riboflavin and UV light treatment effectively reduced the virus titers of human plasma to the limit of detection in tissue culture, regardless of the strain. These data suggest that pathogen reduction in blood products highlight the safety of CP therapy procedures for critically ill COVID-19 patients, while maintaining blood product quality.

1. Introduction

Passive immunotherapy with convalescent plasma (CP) from recovered subjects is an historic therapeutic tool widely used and a pillar of basic immunology [1]. Indeed, the use of immunoglobulins in the prophylaxis and treatment of viral infections is an irreplaceable tool for the post-exposure prevention of several viral infections like VZ and rabies since long time [2]. When a new infectious agent appears passive immunotherapy, is the only weapon available to solve public health emergency. Recently, therapy with convalescent plasma was applied in Western Africa Ebola (2013–2016) and MERS epidemic, (2014–2015) [3]. During the SARS-CoV-1 epidemic caused by a Coronavirus, a 23 % reduction in mortality was reached when convalescent plasma was administered at the early stages of disease [4]. Furthermore, the high levels of safety of plasma therapy was confirmed with the daily transfusion practice of plasma products [5]. At the beginning of COVID-19 pandemic, experts debated whether asymptomatic transmission was possible due to the findings of viral RNA in multiple samples tested (including blood), raising considerable concern regarding the safety of convalescent plasma [6]. Viremia was found in 15 % of patients from Wuhan, China, with a median PCR cycle threshold of 35.1 suggesting a very low RNA copies number [7]. Several reports found viremia in asymptomatic patients, posing a potential risk in blood donation due to the possibility to escape current health screening performed at the time of donation. Starting from March 2020 until now, in response to the outbreak of the SARS-CoV-2 pandemic, convalescent plasma (CP) has

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Abbreviations: SARS-CoV-2, severe acute respiratory syndrome virus 2; COVID-19, coronavirus disease 2019; TCID50, median tissue culture infectious disease; PRT, Pathogen Reduction Technology; CP, convalescent plasma.

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been used for the treatment of severe COVID -19 patients [8] due to the lack of an established therapeutic strategy.

Pathogen reduction systems (PRS) have been also widely implemented to increase the safety of COVID-19 CP, considering that most CP donors have no donation history and thus their donations should be considered at higher infectious risk. The Mirasol Pathogen Reduction Technology (PRT) System (Terumo BCT, Lakewood Co, USA) combines the use of a UV light source and riboflavin (vitamin B2) loading to an irreversible damage to nucleic acids. This system inactivates viruses, bacteria, parasites and white cells, while red blood cells, platelets and plasma proteins are preserved. Our hospital was the first in Italy to use CP for the treatment of critical COVID-19 patients [9,10] during the first pandemic wave in March 2020. In compliance with the recommendations of National authorithy (Centro Nazionale Sangue) we adopted the Mirasol PRT technology to inactivate CP produced at Transusion Service. In the present experimental study, we used infectivity assays to evaluate the reduction of SARS-CoV-2 infectivity after inoculation of different viral variants into plasma samples and subsequently treated by Mirasol PRT system.

2. Methods

2.1. Plasma products

Plasma products were prepared from 25 whole blood units collected in Citrate Phosphate Dextrose (CPD) from regular healthy blood donors, separated on an automated blood system and discarded by local Blood Bank Processing and Validation Center of Fondazione IRCCS Policlinico San Matteo according to the Italian law decree of Ministry of Health, November 2, 2015, "Provisions relating to the quality and safety requirements of blood and blood components". The products were released as PF24 (plasma frozen within 24 h after phlebotomy to \leq -20 °C). After thawing in a water bath at 37 °C, plasma units were pooled and divided in sets of 36 plasma bag units in order to reduce donor variability that might affect the assay's outcome.

2.2. SARS-CoV-2 variants culture protocol

SARS-CoV-2 strains, including wild type Chinese-derived strain (D614), Italian strain PV10734 (D614 G), Alpha strain (501Y.V1 lineage B.1.1.7), Gamma strain (501Y.V3 lineage P.1), Beta strain (501Y.V2 lineage B.1.351) and Delta strain (B.1.617.2) were isolated from infected patients' nasal swabs. The viruses were propagated in Vero E6 cells [VERO C1008 (Vero 76, clone E6, Vero E6, ATCC® CRL-1586™] with the addition of respiratory medium: EMEM plus 1% penicillin, streptomycin, glutamine and 5 γ /mL of trypsin. Mediums were harvested from infected cells, clarified by centrifugation and each strain was titrated and frozen at -80C in aliquots until use. The identity of SARS-CoV-2 strains were established by sequencing [11] and confirmed with a database of sequence data of the viruses, submitted to GISAID under the following reference numbers (EPI_ISL_568579; EPI_ISL_1403609-11). Virus propagation occurred in BSL-3 laboratory. All virus concentration results are presented in median tissue culture infectious doses (TCID₅₀).

2.3. Pathogen reduction study

Pooled plasma derived from blood donors was divided in 36 equal volume into illumination bags (Mirasol Illumination Bag, Terumo BCT, Lakewood, CO). Riboflavin solution for virus inactivation (35 mL) and heparin (Epsoclar, 2500 unit in 0,5 mL, Pfizer NY, US) were added to each bag (230 mL total final volume) to avoid plasma coagulation during microtitration assay. An aliquot of plasma sample was removed from each bag and tested for the presence of specific SARS-CoV-2 IgG and neutralizing antibodies (NT-Abs). In addition, a specific real-time RT-PCRs targeting RNA-dependent RNA polymerase and ORF8 genes was used to detect presence of SARS-CoV-2. In each plasma bag 200 $TCID_{50}$ of virus (6 bags respectively for each strain) was added, according to stock titer. Plasma bags were placed into the Mirasol Illuminator for UV inactivation that consists of exposure to 6.24 J/mL UV light for an average time of 10 min, according to the manufacturer guidelines (https://www.terumobct.com/mirasol). A pre- and post-treatment sample was obtained for viral titer determination with inhouse microtitration assay.

2.4. Microtitration assay

Titration of SARS-CoV2 variants after treatment were defined according to reported protocol [12]. Briefly, 50 μ l of plasma sample from each pool bag were diluted in 50 μ l (1:2) of respiratory medium in two wells of a flat bottom tissue culture microtiter plate (COSTAR, 13 Corning Incorporated, NY 14831, USA) and titrated up to 1:128 in a serial 1:4 dilution. $3x10^4$ VERO E6 cells [VERO C1008 (Vero 76, clone E6, Vero E6); ATCC® CRL-1586TM] in 50 μ l were added and incubated at 33 °C 5% CO2. After 72 h plates were scored for cytopathic effect (CPE), stained with Gram's crystal violet solution (Merck KGaA, 64271 Damstadt, Germany) plus 5% formaldehyde 40 % m/v (Carlo ErbaSpA, Arese (MI), Italy) for 30 min and washed under running water. Blue staining of wells indicated the absence of cytopathic effect. Virus titrations were calculated using Reed-Muench method [13]. Every treated bag was tested in duplicate with each variant.

2.5. Calculation of limit of detection and log reduction

When, in the post treatment samples, no virus was detected in the lowest dilution, the limit of detection for the assay was reached [14]. All values at the limit of detectability of our test were considered less than or equal to the calculated theoretical detection limit. LOD (limit of detection) and log reduction was calculated using the following equations:

 $LOD = \log [1/(N \times V)]$

Log Reduction = Log (Starting Titer) - Log (Final Titer)

N stands for the number of replicas tested at the lowest dilution per sample; V is the volume used for viral titration (volume inoculated/ well in mL). No cytotoxicity occurred at the lowest dilution.

2.6. Quantitative SARS-CoV-2 S1/S2 IgG and neutralizing antibodies (NT-Abs) measurement

Plasma samples were analyzed using a chemiluminescent immunoassay (CLIA) (LIAISON® SARS-CoV-2 S1/S2 IgG; DiaSorin, Saluggia (VC), Italy) for the quantitative characterization of SARS- CoV-2 anti-S1 and anti-S2 IgG antibodies, according to the manufacturer's instructions. Results were given as AU/mL and a cut-off of 15 AU/mL was considered for definition of positive samples. Results ranging from 12 to 15 AU/mL were considered borderline or weak positive and IgG titres <12 AU/mL were given as a negative result.

For SARS-CoV-2 neutralizing antibodies a titer <1:10 was defined as negative whereas a titer >1:10 was considered positive [12].

2.7. SARS-CoV-2 genome detection

Commercial SARS-CoV-2 specific real-time RT-PCRs (MGISP-NE384, MGI Tech Co., Ltd., China) targeting RNA-dependent RNA polymerase and ORF8 genes was performed to detect the presence of SARS-CoV-2 genome in plasma samples collected before virus inoculum and post UV treatment, according to the manufacture guidelines. SARS-CoV-2 RNA amounts are reported as quantification cycle (Cq).

Table 1

Log reduction of SARS-CoV-2 and variants of concern after PRT treatment of pooled plasma units at volume of 230 mL.

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ApplaUNT NUMBERPETRATMENT VIRAL TITRE (Log)POST-TREATMENT VIRAL TITRE (Log)I GO REDUCTOR131.02.1.1.0.02131.42.1.0.02151.48.1.0.02161.40.1.0.02171.41.1.0.02181.49.1.0.0219.13%.0.075%.0.0410.0.075%.0.07.0.0711.18.1.0.0212.13%.1.0.0219.1.02.1.0.0219.1.02.1.0.02200 TOD2019.1.02.0.0219.1.02.0.02.0.0210 TOD2019.0.02.0.0210 TOD201.18.1.0.02200 TOD201.13.1.0.02200 TOD201.13.1.0.0219.1.02.0.02.0.0210 TOD201.13.1.0.02200 TOD201.13.1.1200 TOD201.12.1.12.1200 TOD201.12.1.12.1200 TOD201.12.1.12.1200 TOD201.12.1.12.1.12200 TOD201.13.1.22.1.12200 TOD202.12.1.12.1.12200 TOD201.12.1.12.1.12200 TOD202.12.1.12.1.12200 TOD202.12.1.12.1.12 <td></td> <td>CV</td> <td>1,466%</td> <td>N/A</td> <td>N/A</td>		CV	1,466%	N/A	N/A
Appa 201 TDb,nINT NUMBERRETREATMENT VIRAL TITRE (Log)POST-REATMENT VIRAL TITRE (Log)IO REDUCTION 0.01131.0010.00141.4210.42151.4010.42161.4010.40171.4110.40181.4010.40191.4010.40100.751.401100.75N/AN/A110.75N/AN/A121.1010.10211.1210.10221.1610.12231.1210.12241.1210.13250.029N/A0.12241.1210.12250.029N/A0.12260.299%N/A0.12272.99%11.2282.3111.2292.311.121.12200.299%N/A1.12211.121.121.12211.131.121.12211.141.141.14211.121.12211.141.14211.121.14211.121.14211.121.14211.121.14211.141.14211.141.14211.14<					
UNT NUMBERNETREATMENT VIRAL TITRE (Log)DOST-TREATMENT VIRAL TITRE (Log)READUCTION200 TCDDg13(.4210.4013(.42310.4014310.400.4115(.40310.4016(.40310.4017(.41310.4018(.40310.40average(.48310.400.075NANANA0.075NANANA0.075100.0750.075191.10310.016200 TCD20191.1031191.13310.016210 TCD20191.120.12191.120.120.12200 TCD20190.120.12201 TCD20190.120.12201 TCD20190.120.12201 TCD20100.290.13201 TCD20120.290.13201 TCD20120.290.13201 TCD20120.290.13201 TCD20120.290.13201 TCD20120.290.14201 TCD20120.290.14201 TCD20120.290.14201 TCD20120.210.14201 TCD20120.210.14201 TCD20120.210.14201 TCD20120.210.14 <td></td> <td></td> <td></td> <td></td> <td></td>					
Motion of the second se		UNIT NUMBER	PRETREATMENT VIRAL TITRE (Log)	POST-TREATMENT VIRAL TITRE (Log)	LOG REDUCTION
200 TCDD 0131.6010.60141.4210.42151.4810.48171.4110.41184.4210.494001.4210.414011.420.490.494021.4310.494030.75N/ANA701.33%N/ANA701.121.120.0120 TCD1.91.120.1220 TCD1.21.120.1220 TCD1.21.120.1220 TCD1.21.120.1221 TCD1.120.120.1222 TCD1.131.120.1223 TCD1.131.120.1224 TC1.121.120.1225 TCD0.29%N/ANA20 TCD0.25%0.29%N/ANA20 TCD0.21.33%1.120.1221 TCD1.121.121.120.1222 TCD1.21.121.120.1223 TCD0.20.21.120.1224 TCT1.121.121.1225 TCT0.25%N/ANA20 TCD1.21.121.1220 TCD1.21.121.1220 TCD2.5%0.21.1221 TCT1.121.121.1222 TCT1.121.121.1223 TCT </td <td>Alpha</td> <td></td> <td></td> <td></td> <td></td>	Alpha				
141.421.10.42151.4630.40161.4130.40171.4130.40181.4930.40800.7075N/AN/A800.7075N/AN/A90 <tcd50< td="">11010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.1010.10200<tcd50< td="">1.100.10200<tcd50< td="">1.100.10200<tcd50< td="">1.100.10200<tcd50< td="">1.100.10200<tcd50< td="">1.100.10200<tcd50< td="">1.100.10200<tcd50< td="">1.100.10200<tcd50< td="">1.100.10200<tcd50< td="">1.101.10200<tcd50< td="">1.101.10200<!--</td--><td>200 TCID₅₀</td><td>13</td><td>1,60</td><td>≤ 1</td><td>\geq0,60</td></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<></tcd50<>	200 TCID ₅₀	13	1,60	≤ 1	\geq 0,60
151.48.1.0.0171.41.1.0.0181.49.1.0.0Naverage1.48.1.0.00.0750.075.0.0.0.0Cu5.133%.0.0.0.0200 TODsa19.10.0.0.0201 TODsa19.10.1.0.0201 TODsa19.10.1.0.0201 TODsa112.1.1.0.0201 TODsa1.12.1.1.0.0201 TODsa1.12.1.1.0.0201 TODsa1.12.1.1.1.0201 TODsa1.12.1.1.1.0201 TODsa1.12.1.1.1.1201 TODsa1.13.1.1.1.1201 TODsa2.5.1.1.1.1201 TODsa2.5.1.1.1.1201 TODsa2.5.1.1.1.1201 TODsa2.5.1.1.1.1201 TODsa1.1.1.1.1.1201 TODsa1.1.1.1.1.1201 TODsa2.5.2.1.1.1201 TODsa2.5.2.1.1.1201 TODsa2.5.2.1.1.1201 TODsa <td< td=""><td></td><td>14</td><td>1,42</td><td>≤ 1</td><td>≥0,42</td></td<>		14	1,42	≤ 1	≥0,42
161.405150.40181.443150.49181.483150.49200 TCDDp1.483150.49200 TCDDpUTI NUMBERPETREATMENT VIRAL TITRE (Log)POST-TREATMENT VIRAL TITRE (Log)DG REDUCTION200 TCDDp191.185150.40191.18515150.40200 TCDDp191.185151201 TCDDp1.18515151211.12515151221.16515151231.12515151241.12515151250.029N/AN/AN/A700.299%N/AN/AN/A700.299%N/AN/AN/A702.299%115151200 TCDDp2.22.20515151200 TCDDp2.22.299%115151200 TCDDp100 NUMBERPETREATMENT VIRAL TITRE (Log)PETREATMENT VIRAL TITRE (Log)N/A201 TCDDp2.22.20515151201 TCDDp2.22.20515151201 TCDDp2.22.21515151201 TCDp100 NUMBERPETREATMENT VIRAL TITRE (Log)PETREATMENT VIRAL TITRE (Log)12.22201 TCDp2.212.22515151201 TCDp2.22 <td< td=""><td></td><td>15</td><td>1,48</td><td><1</td><td>>0,48</td></td<>		15	1,48	<1	>0,48
171.415150.41181.495150.49werage1.485150.48CW0.075N/AN/ACWV51.33%N/AN/ACUTODoUTI NUMBERPETERATMENT VIRAL TITRE (Log)POST-TREATMENT VIRAL TITRE (Log)LOG REDUCTON200 TCDo191.105120.10210 TCDo1.125120.10220 TCDo1.125120.10221 UI NUMBER1.125120.12222 UI 1.165120.12232 UI 1.221.1231243 UI 1.225120.13244 UI 1.225120.13254 UI 1.222.39%N/AN/AVUT NUMBERPETERATMENT VIRAL TITRE (Log)POST-TREATMENT VIRAL TITRE (Log)10.038eth0.029N/AN/AN/A200 TCDo2.299%N/AN/AN/A200 TCDo2.299%N/AN/AN/A200 TCDo2.215151.2251.22200 TCDo2.225151.2251.22200 TCDo2.245151.22200 TCDo2.245151.22200 TCDo2.215151.22200 TCDo2.225151.22200 TCDo2.245151.22200 TCDo2.245151.22200 TCDo2.245151.22200 TCDo2.245151.22200 TCDo		16	1.40	- <1	>0.40
18 1.49 -0 -0.09 wrnge 1.49 -0 -0.09 wrnge 0.0075 N/A N/A CV 3.33% N/A N/A CV 3.33% N/A N/A VINT NUMBER PRETREATMENT VIRAL TITRE (Log) POST-TREATMENT VIRAL TITRE (Log) LOG REDUCTION 200 TCDb ₂₀ 19 1.10 -1 -0.10 21 1.12 -1 -0.10 -0.12 21 1.12 -1 -0.10 -0.12 23 1.13 -1 -0.10 -0.12 24 1.12 -1 -0.10 -0.12 24 1.12 -1 -0.13 -0.12 24 1.12 -1 -0.13 -0.12 24 1.12 -1 -1 -0.12 25 2.28 -1 -1 -1.28 200 TCDb ₂₀ 21 -1 -1.23 -1.23 29 2.21 <		17	1 41	<1	>0.41
average SD1.463 130.46SD0.075 0.133%N/AN/AGamma 200 TCDbgUNT NUMBERPETREATMENT VIRAL TITRE (Log)DOST-TREATMENT VIRAL TITRE (Log)LOG REDUCTONDGamma 200 TCDbg191.105130.10200 TCDbg191.105130.10210 TCDbg1.125130.12220 TCDbg1.135130.12241.125130.12251.135130.12241.125130.12250.029N/AN/A200 TCDbg0.29N/AN/A200 TCDbg1.135130.12200 TCDbg2.59%N/A30.12200 TCDbg2.59%N/A30.12200 TCDbg2.59%131.22200 TCDbg2.59%131.22200 TCDbg2.502.3251200 TCDbg2.512.3251200 TCDbg2.512.3251200 TCDbg512.3431.22200 TCDbg513.043.12200 TCDbg513.043.12200 TCDbg513.043.12200 TCDbg513.043.12200 TCDbg513.043.12200 TCDbg513.043.12200 TCDbg513.043.12200 TCDbg513.143.12200 TCDbg513.143		18	1 49	<1	>0.49
Intrage $1, 0^{0}$ NA NA NA NA CV 5, 000 $0,075$ NA NA NA CUT PRETREATMENT VIRAL TITRE (Log) POST-TREATMENT VIRAL TITRE (Log) LOG REDUCTION 200 TCDD ₂₀ 19 $1,10$ ≤ 1 $= 0,10$ 21 $1,12$ ≤ 1 $= 0,10$ 23 $1,13$ ≤ 1 $= 0,10$ 23 $1,12$ ≤ 1 $= 0,10$ 23 $1,12$ ≤ 1 $= 0,13$ 24 $1,12$ ≤ 1 $= 0,13$ 24 $0,029$ NA NA 30 $0,029$ NA NA 30 $0,299$ NA NA 200 TCDD ₂₀ 25 $2,28$ 21 $21,23$ 200 TCDD ₂₀ 25 $2,28$ 21 $21,23$ 200 TCDD ₂₀ $21,23$ $21,23$ $21,23$ $21,23$ 200 TCDD ₂₀ $21,23$		average	1 49	<1	>0.48
BD CV S133% N/A N/A Gamma UNT NUMBER PRETREATMENT VIRAL TITRE (Log) POST-TREATMENT VIRAL TITRE (Log) LOG REDUCTION 200 TCDD ₂₀ 19 1,10 1 20,10 21 1,10 1 20,10 20,10 22 1,16 1 20,12 20,12 23 1,12 1 20,12 20,16 24 1,12 1 20,12 20,16 25 1,21 1 20,12 20,12 34 1,22 1,13 20,12 20,12 34 0,029 NA NA NA Vertarge 1,13 21 20,12 20,13 35 0,029 NA NA NA VCV 2,59% NA NA NA 200 TCDD ₂₀ 25 2,28 1 21,28 200 TCD ₂₀ 25 2,21 2,41 21,20 200 TCD ₂₀ 2,44		average SD	0.075		≥0,48 N (A
CurrCurrFurtherNANA200 TCDD00UNIT NUMBERPERTEATMENT VIRAL TITRE (Log)POST-TREATMENT VIRAL TITRE (Log)LOG REDUCTION200 TCDD00191,10 \leq \geq 0,102011,18 \leq \geq 0,122011,16 \leq \geq 0,122021,16 \leq \geq 0,12211,12 \leq \geq 0,12221,13 \leq \geq 0,12241,12 $<$ \geq 0,12241,12 $<$ \geq 0,122500,029N/AN/ACV2599%N/AN/AVITT NUMBERPERTEATMENT VIRAL TITRE (Log)POST-TREATMENT VIRAL TITRE (Log)LOG REDUCTIONBeta201 CCDD022,22 \leq $<$ $<$ 200 TCDD025,022,18 $<$ $<$ $<$ 200 TCD02,18 $<$ $<$ $<$ $<$ 200 TCD02,22 $<$ $<$ $<$ $<$ 200 TCD02,23 $<$ $<$ $<$ $<$ 200 TCD02,21 $<$ $<$ $<$ $<$ 200 TCD02,22 $<$ $<$ $<$ $<$ 200 TCD02,18 $<$ $<$ $<$ $<$ 200 TCD02,18 $<$ $<$ $<$ $<$ 200 TCD02,23 $<$ $<$ $<$ $<$ 200 TCD02,03 $<$ $<$ $<$ $<$ 200 TCD02,04 $<$ $<$ $<$ $<$		SD	0,075	N/A	N/A
Gamma 200 TCD200UTI NUMBERPRETREATMENT VIRAL TITRE (Log)POST-TREATMENT VIRAL TITRE (Log)LOC REDUCTION191.1010.100.11201.1210.110.11211.1210.110.11221.1610.0160.11231.1310.0120.012241.1210.0120.012252.021.1310.012202.030.020N/A0.012241.120.0120.0120.012252.09%N/AN/A0.012202.59%N/AN/A0.012202.59%N/A11.22202.2211.22202.2311.22202.2411.23212.2211.23212.2211.24202.121.12202.121.12212.141.12212.141.12222.141.12232.121.12241.141.12252.141.12262.12272.141.12282.021292.14202.14202.14212.12212.14222.12232.14242.14		CV	5,133%	N/A	N/A
Gama 201 TCD20INT NUMBERPRETRATMENT VIRAL TITRE (Log)POST-TRATMENT VIRAL TITRE (Log)IO REDUCTION201 CD201,1010.010.01201 1,1210.010.01201 1,1310.010.01201 1,1310.010.01201 1,1310.010.01201 1,1310.010.01201 1,1310.010.01201 1,1310.010.01201 1,1310.010.01201 1,130.029N/AN/A201 CD200.299N/AN/A201 CD20299%N/A1201 CD202000.029N/A201 CD2020011.02201 CD20212.021201 CD202.2211.02201 CD202.2211.02201 CD202.2211.02201 CD202.2011.21202 CD202.2211.21203 CD202.2411.21204 CD201.241.21205 CD201.544%N/A205 CD201.544%1.21205 CD201.544%1.21205 CD201.544%1.21205 CD201.544%1.21205 CD201.544%1.21205 CD201.544%1.21205 CD201.544%1.21205 CD201.544%1.21205 CD201.544					
Carma Former formation Former formation Former formation Former formation 200 TCD ₉₀ 19 1,10 1 20,10 200 1,18 51 20,18 21 1,12 51 20,12 22 1,16 51 20,16 23 1,13 51 20,13 24 1,12 51 20,13 30 0,029 N/A NA SD 0,029 N/A NA CV 2,59% N/A NA 200 TCD ₅₀ 25 2,28 51 21,28 200 TCD ₅₀ 25 2,28 51 21,28 200 TCD ₅₀ 25 2,28 51 21,28 200 TCD ₅₀ 25 2,28 51 21,23 200 TCD ₅₀ 25 2,23 51 21,23 200 TCD ₅₀ 2,33 51 21,24 30 2,32 51 21,24 2		UNIT NUMBED	DEFTERATMENT VIEAL TITER (Log)	DOST TREATMENT VIRAL TITRE (Log)	LOC REDUCTION
Summa 19 1,10 ≤1 0,10 200 TCD500 19 1,18 ≤1 ≥0,18 21 1,12 ≤1 ≥0,16 22 1,16 ≤1 ≥0,16 23.3 1,13 ≥0,13 ≥0,12 24 1,12 ≤1 ≥0,13 24 1,12 ≤1 ≥0,13 5D 0,029 N/A NA SD 0,029 N/A N/A CV 259% N/A N/A SD 0,029 N/A N/A CV 259% N/A N/A CV 228 ≤1 1 200 TCD50 25 2,28 ≤1 1 212 2,28 ≤1 2.123 1 213 213 1 2.123 1 214 20 2.13 1 1.8 29 2,21 1 1.8 2.12 200 TC	Gamma	ONTI NOMBER	TREIREATIMENT VIRAL TITRE (105)	1001-IREALIMENT VIRAL IIIRE (LOG)	LOG KEDUCIION
200 TCDs 1,10 51 20,0 20 1,18 51 20,18 21 1,12 51 20,12 22 1,16 51 20,16 23 1,13 51 20,13 24 1,12 20 20,13 30 0,029 N/A 20,13 SD 0,029 N/A N/A VINT NUMBER PRETREATMENT VIRAL TITRE (Log) POST-TREATMENT VIRAL TITRE (Log) N/A 200 TCD50 25 2,28 51 21,28 26 2,231 51 21,28 21,28 27 2,18 21,23 21,21 21,23 29 2,21 51 21,23 21,21 30 2,32 51 21,22 21,23 90 2,32 51 21,23 21,23 90 2,32 51 21,23 21,24 90 2,32 51 21,22 21,24	200 TCID	10	1 10	<1	>0.10
200 1,16 5-1 20,12 21 1,12 5-1 20,16 22 1,13 5-1 20,16 23 1,13 5-1 20,16 24 1,12 5-1 20,13 24 1,12 5-1 20,13 24 1,12 5-1 20,13 24 1,12 5-1 20,13 350 0,029 N/A NA CV 2,599% N/A NA V 2,599% N/A NA VA NA NA NA VA 2,599% N/A NA VA NA NA NA VA 2,599% N/A NA VA 2,20 5-1 1,28 26 2,23 5-1 1,21 27 2,18 5-1 1,22 28 2,22 5-1 1,22 90 2,24 1	200 1 CID ₅₀	19	1,10		>0.10
1 1,12 2,1 20,16 20,16 23 1,13 21 20,13 24 1,12 21 20,13 average 1,13 21 20,13 SD 0,029 N/A 20,13 SD 0,029 N/A N/A CV 2599% N/A N/A 200 TCDbyo 25 2,28 21 21,28 26 2,23 21 21,28 21,28 27 2,18 21,21 21,20 21,21 28 2,20 21 21,21 21,21 29 2,21 21 21,21 21,21 30 2,23 11 21,22 21,22 30 0,34 N/A N/A 12,21 200 TCDbyo 1 14/4 14,21 12,22 200 TCDbyo 1,54 1 20,71 12,22 200 TCDbyo 1,54 1 20,71 12,22 <td></td> <td>20</td> <td>1,18</td> <td>≤ 1</td> <td>≥0,18</td>		20	1,18	≤ 1	≥0,18
24 1,10 1 2010 20,10 23 1,13 1 20,13 20,13 24 1,12 1 20,13 30 0,029 N/A N/A SD 0,029 N/A N/A V 259% N/A N/A 200 TCID ₅₀ 25 2,239% SD 20,02 200 TCID ₅₀ 25 2,28 1 21,28 200 TCID ₅₀ 2,2 2,23 1 21,23 27 2,18 1 21,23 21,23 28 2,02 1 20,02 21,23 28 2,02 1 21,21 20,02 21,21 30 2,21 3 1,20 21,21 20,02 21,21 30 2,22 3 1 20,02 21,21 20,02 21,21 20,02 21,21 20,02 20,02 21,21 20,02 20,02 21,22 20,02 20,02		21	1,12	≤ 1	≥0,12
23 1,13 ≤ 1 $\geq 0,13$ 24 1,12 ≤ 1 $\geq 0,13$ average 1,13 ≤ 1 $\geq 0,13$ BD 0,029 N/A N/A CV 2,59% N/A N/A Dete PRETREATMENT VIRAL TITRE (Log) PST-TREATMENT VIRAL TITRE (Log) LOG REDUCTION Beta 2 2,28 ≤ 1 $\geq 1,28$ 26 2,28 ≤ 1 $\geq 1,28$ 27 2,18 $\leq 1,21$ $\geq 1,20$ 28 2,20 ≤ 1 $\geq 1,23$ 30 0,223 $\leq 1,21$ $\geq 1,20$ 29 2,21 ≤ 1 $\geq 1,23$ 30 2,22 ≤ 1 $\geq 1,23$ average 2,22 ≤ 1 $\geq 1,20$ 20 0,034 N/A $> 1,22$ Average 1,544% $> NA$ $> 0,71$ 200 TCD ₅₀ 31 $1,71$ ≤ 1 $\geq 0,71 21,22 > $		22	1,16	≤ 1	≥0,16
24 1,12 ≤1 ≥0,12 average 1,13 ≤1 ≥0,13 SD 0,029 N/A N/A CV 2,599% N/A N/A UNIT NUMBER PRETREATMENT VIRAL TITRE (Log) POST-TREATMENT VIRAL TITRE (Log) LOG REDUCTION Beta 200 TCID ₅₀ 25 2,28 ≤1 21.28 200 TCID ₅₀ 25 2,28 ≤1 21.28 27 2,18 ≤1 21.28 28 2,20 ≤1 21.28 29 2,21 ≤1 21.23 29 2,21 ≤1 21.23 29 2,21 ≤1 21.23 30 2,22 ≤1 21.21 30 2,22 ≤1 21.22 SD 0,034 N/A N/A CV 1,544% N/A 20.71 200 TCID ₅₀ 31 1,76 ≤1 20.76 33 1,74 ≤1 <td< td=""><td></td><td>23</td><td>1,13</td><td>≤ 1</td><td>$\geq 0,13$</td></td<>		23	1,13	≤ 1	$\geq 0,13$
average 1,13 ≤ 1 $\geq 0,13$ SD 0,029 N/A N/A CV 2,599% N/A N/A Beta UNT NUMBER PETREATMENT VIRAL TITRE (Log) POST-TREATMENT VIRAL TITRE (Log) LOG REDUCTION Beta 200 TCID ₅₀ 25 2,28 ≤ 1 $\geq 1,28$ 200 TCID ₅₀ 25 2,28 ≤ 1 $\geq 1,28$ 200 TCID ₅₀ 25 2,28 ≤ 1 $\geq 1,28$ 200 TCID ₅₀ 25 2,28 ≤ 1 $\geq 1,28$ 200 TCID ₅₀ 25 2,21 ≤ 1 $\geq 1,28$ 200 TCID ₅₀ 2,21 ≤ 1 $\geq 1,28$ 200 TCID ₅₀ 2,23 ≤ 1 $\geq 1,22$ 30 2,23 ≤ 1 $\geq 1,22$ 400 TCID ₅₀ V/A N/A N/A CV 1,544% N/A N/A 200 TCID ₅₀ 31 1,71 ≤ 1 $\geq 0,71$ 212 1,54 ≤ 1		24	1,12	≤ 1	\geq 0,12
BD 0,029 N/A N/A V 2,599% N/A N/A 200 TCD ₅₀ PRETREATMENT VIRAL TITRE (Log) POST-TREATMENT VIRAL TITRE (Log) LOG REDUCTION 200 TCD ₅₀ 2,5 2,28 51 2,128 200 TCD ₅₀ 2,5 2,23 51 2,123 27 2,18 51 2,121 28 2,21 51 2,121 29 2,21 51 2,123 29 2,21 51 2,123 30 2,22 51 2,123 30 2,23 51 2,123 30 2,23 51 2,123 30 2,23 51 2,123 30 2,23 51 2,123 30 2,32 51 2,123 30 2,32 51 2,123 30 3,13 1,71 51 200 TCD ₅₀ 31 1,71 51 31 1,71 51 2,071 32 1,54 51 2,054 31 1,71 51 2,071 32 1,47 11 2,071 33 1,64 2,123 <td></td> <td>average</td> <td>1,13</td> <td>≤ 1</td> <td>\geq0,13</td>		average	1,13	≤ 1	\geq 0,13
CV 2,599% N/A N/A Beta		SD	0,029	N/A	N/A
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average 1,59 ≤ 1 $\geq 0,59$ SD 0,207 N/A N/A CV 13,010% N/A N/A		36	1,4/	51	≥0,47
SD 0,207 N/A N/A CV 13,010% N/A N/A		average	1,59	<u>≤1</u>	≥0,59
CV 13,010% N/A		SD	0,207	N/A	N/A
		CV	13,010%	N/A	N/A

SARS-CoV-2 and variants viral titer reduction after pathogen reduction treatment (PRT). Each condition was tested in duplicate, including two replicas for each bag strains group. Pooled plasma bags were inoculated with a known quantity of coronavirus and each one was treated independently. After virus inoculum titration occurred before and after PRT treatment. TCID50 = median tissue culture infectious dose. 200 TCID50 Is the stock inoculum value, Log viral titer is the higher dilution number of inoculated samples that can produce observable cytopathic effect, $\log \leq 1$ mean no cytopathic in the lowest dilution.

Table 2

Sars-CoV-2 specific real-time RT-PCRs quantification cycle (CQ) in pooled plasma.

sample after PR	Т					
	Sars-CoV-2	specific real	-time RT-PO	CRs quantific	ation cycl	le (CQ)
Post UV treatment plasma Bag N#	6	10	14	19	25	32
ORF8 Gene (CQ)	28	28	28	31,8	26	26,9
RdRp Gene (CQ)	27,6	27,7	28	31	25	27,1
Strain	ITALIAN	CHINESE	ALPHA	GAMMA	BETA	DELTA

Quantification cycles of SARS-CoV-2 variants is reported as aquantification cycle (CQ) in six pooled plasma bags, analysed post pathogen reduction treatment (PRT).

2.8. Data analysis

All analyses reported including descriptive statistics for pathogen reduction were performed using GraphPad Prism software (version 5; GraphPad Software Inc., La Jolla, CA), including mean, standard deviation, coefficient of variation (CV) and numbers of samples analyzed (N).

3. Results

Thirty-six (N = 36) PF24 pooled plasma units were evaluated using Mirasol PRT system against SARS-CoV-2 and its variants of concern. In order to exclude prior virus neutralization by immune components in the plasma products, we performed a quantitative SARS-CoV-2 S1/S2 IgG and NT-Abs detection that tested negative. Moreover, the investigation by a SARS-CoV-2 specific real-time RT-PCRs for the presence of viral genome in plasma bags before inoculation of the different variants, was negative.

The data collected are shown in Table 1. The in vitro microtitration assay demonstrated that the pathogen reduction treatment of plasma bags inoculated with SARS-CoV-2 was able to reduce infectious titer to the limit of detection by $\geq 1,21 \log$ for Italian, $\geq 1,13 \log$ for Chinese, $\geq 0,48 \log$ for Alpha, $\geq 0,13 \log$ for Gamma, $\geq 1,22 \log$ for Beta and $\geq 0,59 \log$ for Delta strain. Indeed all thirty-six treated units were reduced to the limit of detection ($\leq 1 \log 10$). All samples were tested immediately before and after Mirasol treatment. Furthermore, we tried to isolate the viral strains after treatment, inoculating plasma samples on VERO E6. Despite the high copies number detected by RT-PCR for each viral strain (Table 2) after treatment, viruses were completely inactivated and not able to infect VERO E6 cells (Table 3).

4. Discussion

Convalescent plasma, a source of anti-SARS-CoV-2 antibodies, has been widely used as a treatment for COVID-19 during the present pandemic. The safe use of CP depends mainly on the accuracy in donor screening for transmitted transfusion diseases, potentially harmful for the recipient, considering that many CP donors could be first time donors [15].

The Mirasol PRT treatment of CP is a safe method that significantly reduces the risk of possible transmitted transfusion infections and, importantly, does not impair plasma quality (e.g.:antibody function [16]). In the study by Keil et al., the Mirasol PRT system significantly reduced the load of viral agents tested in plasma and platelet products [17]. This method provides a proactive layer of blood safety through its broad-based effectiveness against a wide range of known pathogens.

The European Center for Disease Prevention and Control (ECDC) suggests a precautionary deferral from blood donation for 21 days after any possible exposure for confirmed COVID-19 patients [18]. Despite SARS-CoV-2 virus was detected at very low levels in blood products [19] and there is no evidence of transfusion transmission at this time, the theoretical and potential risk of SARS-CoV-2 and their variants of concern to be transmitted through transfusion is unknown. Recently Shawn et al. reported the efficacy of Mirasol PRT system in reducing SARS-CoV-2 (Chinese derived isolate, USA-WA1/2020) strain in plasma and platelets without impairing product quality e.g.: antibody function [20]. During a pandemic, pathogen reduction approach may provide an important first line of defense against the transfusion transmission of an unknown outbreaking agent along with reducing residual risk of co-infections.

SARS-CoV-2 is a pathogen characterized by frequent mutations since its first isolation in December 2019, prone to develop different strains that can escape immunity. Our results show that riboflavin and UV light effectively inactivate different variants of SARS-CoV-2 as demonstrated by the results of the in vitro microtitration assay. These data confirm previous results obtained with MERS-CoV [21], suggesting that Mirasol system is effective against different coronaviruses. Furthermore, the comparison between the results of molecular assay after plasma inactivation with Mirasol system and virus isolation on VERO E6 show that the Mirasol PRT system is a safe and reliable method for the inactivation of Sars-CoV-2 contamination, highlighting the safety of CP therapy procedures for critically ill COVID-19 patients, maintaining the plasma quality.

5. Conclusion

Pathogen reduction Riboflavin and UV light treatment greatly reduced the virus titer of SARS-CoV-2 and its variants in human plasma, resulting in inactivated viruses unable to infect tissue culture and consequently not transmittable through transfusion. Although the risk of

Table 3

Viral isolation from pooled plasma sample after PRT.

	Sars-CoV-2 plasma isolation results					
Post UV treatment plasma Bag N#	6	10	14	19	25	32
virus isolation result Strain	negative ITALIAN	negative CHINESE	negative ALPHA	negative GAMMA	negative BETA	negative DELTA

Viral genome detected in pooled plasma after pathogen reduction treatment (PRT) is not able to infect VERO E6 cells. At 120 h post incubation with VERO E6 cells, plates were scored and showed no cytopathic effect (CPE) due to inactivation of the viral genome.

viral transfusion transmission is suspected to be low, implementation of pathogen reduction technology might result in a better protection of plasma transfusion recipients during this and future pandemic.

Data availability

Data will be made available on request.

The data that has been used is confidential.

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Authorship contributions

Conceptualization, P.C., E.P., C.D.F. and A.F.; methodology, A.F., C. D.F, J.C.S., and A.F.; software, A.F. and C.M.; formal analysis, S.D.V. and E.B.;; resources, F.B.; data curation, A.F. and I.C.; writing—original draft preparation, A.F. and E.P.; writing—review and editing, E.P., I.C., P.C., C.D.F. and F.B.; visualization, F.B.; investigation, D.T. and F.P.; funding acquisition, F.B. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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