Journal of proteome research

pubs.acs.org/jpr

Technical Note

Inactivation of Coronaviruses during Sample Preparation for Proteomics Experiments

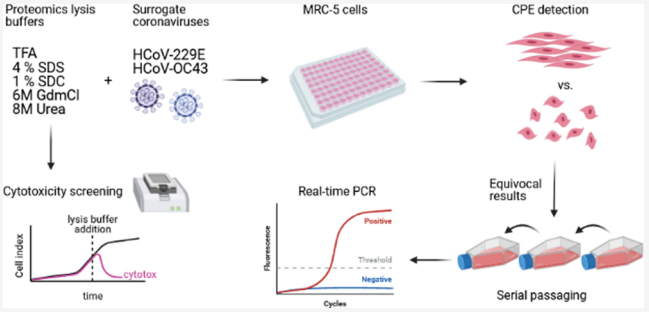
Marica Grossegesse,*,# Paula Leupold,# Joerg Doellinger, Lars Schaade, and Andreas Nitsche

Cite This: *J. Proteome Res.* 2021, 20, 4598–4602

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Mass spectrometry-based proteomics is applied in SARS-CoV-2 research and is, moreover, being discussed as a novel method for SARS-CoV-2 diagnostics. However, the safe inactivation of coronaviruses by proteomics lysis buffers has not been systematically analyzed yet. Hence, for safety reasons a heating step prior to sample preparation is often performed. This step could be omitted once the safe inactivation with the typical buffers is proven. Here we test five different proteomics lysis buffers—4% SDS, 1% SDC, TFA, 6 M GdmCl, and 8 M urea—for their inactivation capacity of coronaviruses. Two representative human coronaviruses, namely HCoV-229E and HCoV-OC43, were used as surrogate for SARS-CoV-2. Lysis was performed at room temperature and at 95 °C for 5 min. Inactivation was confirmed by the absence of a cytopathic effect in MRC-5 cells, and equivocal results were further confirmed by serial passaging and quantitative real-time PCR. While at room temperature SDS, SDC, and TFA inactivated both coronaviruses, and GdmCl and urea resulted in partially incomplete inactivation. This demonstrates that care should be taken when choosing lysis buffers for proteomics analysis of coronaviruses, because some buffers do not ensure inactivation and, hence, biosafety during the further sample preparation.



KEYWORDS: *coronavirus, inactivation, sample preparation, proteomics, biosafety*

INTRODUCTION

In December 2019, the first cases of severe pneumonia of unknown origin were reported in Wuhan, China. Shortly afterward, a novel coronavirus was discovered as being the causative agent and named SARS-CoV-2 and the related disease was called COVID-19. The virus turned out to be highly contagious, leading to a still ongoing pandemic with already more than 2.7 million deaths worldwide. Mass spectrometry (MS)-based proteomics is a valuable method to elucidate biological characteristics of the novel virus,[1,2] and moreover, MS is being discussed as a diagnostic method to detect SARS-CoV-2 in patient samples.[3−6] SARS-CoV-2 is categorized as a risk group 3 pathogen, and for virus propagation a biosafety level (BSL) 3 laboratory is required according to the WHO laboratory biosafety guidance related to COVID-19. In contrast, nonpropagative diagnostics can be done in a BSL-2 laboratory.[7] Nevertheless, whether working in a BSL-2 or BSL-3 laboratory, the sample has to be transferred out of the laboratory for further sample preparation and MS analysis. Hence, it is crucial to safely inactivate the virus before leaving the high containment laboratory.[4] It is known that SARS-CoV-2 can be inactivated by heat,[8,9] e.g. heating to 65 °C prior to MS sample preparation of respiratory specimens has been applied.[3,5] Although sample preparation for MS-based proteomics often includes a heating step—either during lysis itself or during the reduction/alkylation step—it is not known whether lysis buffers for proteomics are sufficient to inactivate coronaviruses. Therefore, this study aims at analyzing the inactivation efficiency of common proteomics lysis buffers for coronaviruses. We here focus on liquid samples because generally most coronavirus diagnostic samples are oro- or nasopharyngeal swabs in liquid, e.g. transport medium or PBS. The following five lysis buffers were analyzed: 4% sodium dodecyl sulfate (SDS),[3,10] 1% sodium deoxycholate (SDC),[11,12] trifluoroacetic acid (TFA) at a 1:4 ratio,[13,14] 6 M guanidinium chloride (GdmCl),[15] and 8 M urea.[16] All buffers were tested at room temperature (RT) and at 95 °C for 5 min by using low pathogenic human coronaviruses OC43 (HCoV-OC43) and 229E (HCoV-229E) as representative coronaviruses which can be handled in a BSL-2 laboratory.

Received: April 19, 2021
Published: August 25, 2021

MATERIAL AND METHODS

Cell Culture and Virus Propagation

Human lung fibroblasts (MRC-5, ATCC CCL-171) were cultured in Eagle's Minimal Essential Medium (EMEM), supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 1 × MEM nonessential amino acids at 37 °C in a humidified atmosphere of 5% CO_2. HCoV-OC43 (ATCC VR1558) and HCoV-229E (ATCC VR740) were propagated in MRC-5 cells at 35 °C by using T175 cell culture flasks and 30 mL of medium. After 3 days (HCoV-229E) or 6 days (HCoV-OC43), the supernatant was stored at −80 °C until further use.

Virus Titration

Tissue Culture Infection Dose 50 ($TCID_{50}$) assays were performed in 96-well plates to determine the viral titers of HCoV-229E and HCoV-OC43 from cell culture supernatants. Virus stocks were diluted in MRC-5 cell culture medium to form a 10-fold dilution series ranging from 10^{-1} to 10^{-10} and added to MRC-5 cell monolayers (2×10^4 cells/well). Samples were incubated for 4 days at 35 °C. All wells were examined for cytopathic effect (CPE), and the $TCID_{50}$ was calculated according to Reed and Muench.[17] HCoV-229E stock had a titer of 3.2×10^6 $TCID_{50}$/mL, and HCoV-OC43, of 7.9×10^6 $TCID_{50}$/mL. The viral titer in plaque forming units per mL (PFU/mL) was approximated by Poisson distribution assuming that PFU/mL equals $0.7 \times TCID_{50}$/mL.[18]

Assessment of Lysis Buffer Cytotoxicity

The cytotoxicity of different proteomics lysis buffers on MRC-5 cells was determined with the xCELLigence Real-time cell analysis (RTCA) system (Roche, Penzberg, Germany) and the associated software RTCA v2.0. In principle, cells are seeded in a 96-well E-Plate with electrodes at the bottom and the impedance is monitored over time. The cell index (CI) as the output represents the background-corrected impedance and increases with higher confluence and degree of attachment of the cells to the bottom of the wells. A decrease of the CI after addition of the lysis buffer indicates a cytotoxic effect. A background measurement was performed with 50 μL of prewarmed MRC-5 cell culture medium. Subsequently, a total of 10^4 MRC-5 cells in 50 μL of medium were added to each well. The CI measurement was started, and cells were incubated for 2 days. The measurement was paused to add 100 μL of the respective lysis buffer dilutions to the wells (in quadruplicate), resulting in a dilution series ranging from 10^{-1} to 10^{-4} for each lysis buffer. 4% SDS, 1% SDC, 6 M GdmCl, and 8 M urea lysis buffer were prepared in 100 mM Tris, pH 7.6. TFA was neutralized with 2 M Tris at a 1:10 ratio. The lysis buffers were diluted with MRC-5 cell culture medium by a factor of 5 and sterile filtered, and three further 10-fold dilutions were prepared. For the negative control, 100 μL of MRC-5 cell culture medium were added. The E-plate was placed back into the station, and CI measurement was continued for 4 days. Note that the measurement is temperature sensitive, and a temporary decrease in CI is observed after removal and reinsertion of the plate. The data output was normalized to the time point of lysis buffer addition, and the temperature-dependent decrease was excluded from the final data set.

Virus Inactivation

HCoV-OC43 and HCoV-229E virus stocks were used to test coronavirus inactivation with proteomics lysis buffers, heat, and combinations thereof. Inactivation was performed in triplicate for each lysis buffer−temperature combination. To ensure infectivity of the virus stocks, back-titration by using the $TCID_{50}$ assay was performed simultaneously as described above. Lysis buffers were prepared as described for the cytotoxicity assay. TFA was neutralized with 2 M Tris. The virus stock solutions were mixed with lysis buffers (1:4), briefly vortexed, and incubated at RT or at 95 °C for 5 min. The mixtures were diluted with MRC-5 cell culture medium to a noncytotoxic concentration, and 100 μL per well were added to prepared cells (2×10^4 MRC-5 cells/well in 100 μL of cell culture medium in a 96-well plate). Each of the potentially inactivated triplicates was used for eight wells. CPE was monitored for 6 days as an indicator for inactivation success. For samples exhibiting ambiguous morphological structures, 200 μL of the supernatant were used in three consecutive blind passages. Passaging was performed at 4 days post infection (dpi), and 1.5×10^6 MRC-5 cells in T25 cell culture flasks were used for each passage. After the third passage, supernatants were collected for real-time PCR analysis.

RNA extraction was performed with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions, except for using 60 μL of nuclease-free water instead of AVE buffer for RNA elution. Real-time PCR targeting HCoV-229E or HCoV-OC43 was performed by using the primers and probes in Table S1 and the pipetting scheme and thermal profile shown in Tables S2 and S3. Each sample was measured in duplicate on a CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, California, USA). Diluted RNA extracts from HCoV-OC43 and HCoV-229E supernatants were used as positive controls. Limits of detection (LOD_{95}) of the real-time PCR assays targeting HCoV-OC43 and HCoV-229E are 1.5 and 6.1 copies per reaction, respectively, as determined by using in vitro RNA.

RESULTS AND DISCUSSION

Cytotoxicity of Proteomics Lysis Buffers

First, we analyzed the cytotoxicity of proteomics lysis buffers on MRC-5 cells to enable the inoculation of cells for the determination of the inactivation success by CPE monitoring. 10-fold dilution series of proteomics lysis buffers, namely 4% SDS, 1% SDC, TFA 1:4, 6 M GdmCl, and 8 M Urea, were used. The dilutions were added to MRC-5 cell cultures of low confluence, and the CI was measured over time by using RTCA. The 10^{-1} dilutions of all five lysis buffers reduced the CI to zero upon addition, demonstrating a clear cytotoxic effect (Figure 1). The same was observed for the 10^{-2} dilutions of the SDS (0.04% final concentration (f.c.)) and GdmCl (80 mM f.c.) lysis buffers. All other dilutions showed no long-lasting reduction of the CI. Some samples, namely the 10^{-2} dilution of SDC (0.01% f.c.), TFA (1:400 f.c.), urea (80 mM f.c.), and the 10^{-3} dilution of GdmCl (6 mM f.c.), induced a temporary decrease of at least 0.1 units which might indicate a slight cytotoxic or growth inhibitory effect. However, the CI increased steadily thereafter. Based on these results, the 1% SDC, TFA, and 8 M urea lysis buffers were considered to be noncytotoxic in MRC-5 cell culture after 100-fold dilution, while the 4% SDS and 6 M GdmCl buffers need to be diluted 1000-fold before they can be applied to the cell monolayer.

https://doi.org/10.1021/acs.jproteome.1c00320
J. Proteome Res. 2021, 20, 4598−4602

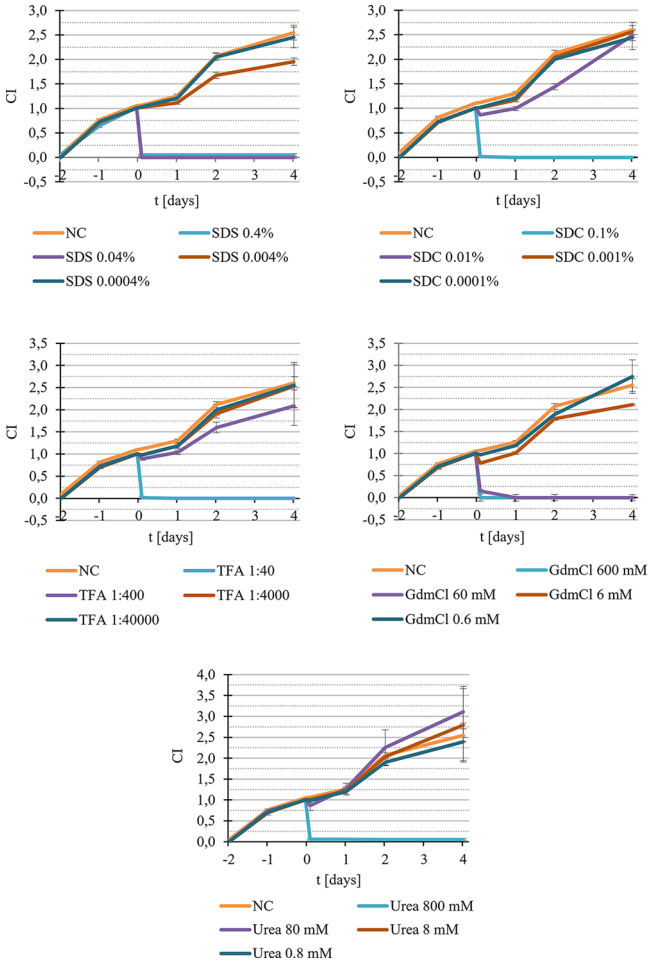


Figure 1. Analysis of cytotoxic effects of proteomics lysis buffers in MRC-5 cells by using Real-time cell analysis (RTCA). MRC-5 cells were grown for 2 days before serial dilutions of different lysis buffers were added at $t = 0$. Cell indices (CI) were monitored as an indicator for cell growth and the degree of attachment in the xCELLigence RTCA system. A decrease in CI is the result of decreasing confluence or degree of attachment of the cell monolayer induced by the respective buffer dilution. Error bars show the standard deviation of at least triplicates. GdmCl: guanidinium chloride. NC: negative control. SDC: sodium deoxycholate. SDS: sodium dodecyl sulfate. TFA: trifluoroacetic acid.

Inactivation of HCoV-OC43 and HCoV-229E by Proteomics Lysis Buffers

The inactivation success of coronaviruses by proteomics lysis buffers was analyzed using virus-induced CPE in MRC-5 cells. HCoV-229E and HCoV-OC43 were used as surrogate coronaviruses. The application of surrogate viruses with similar biophysical properties is a common method in inactivation studies to enable working under lower biosafety level conditions. For example, mouse hepatitis virus (MHV), transmissible gastroenteritis virus (TGEV), and HCoV-229E have been used as surrogate coronaviruses for SARS-CoV and SARS-CoV-2 to analyze the inactivation efficiency of disinfectants on surfaces.[19,20] Moreover, a defined set of model surrogate viruses is used in Europe to test chemical disinfectants applied in human medicine, for example as described in the German guidelines of the DVV/RKI.[21]

In the present study, virus stock solutions were diluted over the course of inactivation and sample preparation. Hence, we first calculated the virus titers used for the final inoculation, resulting in titers between 5.5×10^2 and 1.4×10^4 (Table S4). The final viral titers of all samples were at least 1 order of magnitude higher than the lowest virus concentration for which a CPE could be observed in the $TCID_{50}$ assay. Hence, the lack of a CPE could be considered to be a consequence of virus inactivation. CPE detection in combination with real-time PCR from the supernatant is a standard method applied to test for inactivation of coronaviruses.[22−24]

A clear CPE could only be observed in the positive controls containing noninactivated virus. In contrast, most inactivation samples showed no signs of a CPE. This was true for all SDS-, SDS/heat-, and the heat only inactivated samples. In agreement with the literature, heating for 5 min at 95 °C[9] led to complete inactivation in all samples. However, heating is known to induce carbamylation of proteins in urea-containing samples and, hence, is not recommended for all tested lysis buffers.[25] Regardless of the incubation temperature at which inactivation was performed, the SDC, TFA, and GdmCl samples were generally less confluent than the negative control. While SDC-treated samples showed no sign of a CPE, the GdmCl-treated samples showed CPE-like morphological changes in up to three wells for both viruses. However, this observation was only true for samples incubated at RT but not at 95 °C. Ambiguous results were also observed in two of the urea-treated HCoV-OC43 samples at RT. Interestingly, up to two HCoV-229E wells treated with TFA displayed ambiguous structures, regardless of incubation temperature (Figure 2).

In the current SARS-CoV-2 pandemic, MS-based proteomics is applied to elucidate, for example, virus−host interactions,[26,27] post-translational modifications of viral proteins,[2,28] and biomarkers.[12,29] Moreover, MS has been suggested as an alternative method for SARC-CoV-2 diagnostics and used to detect SARS-CoV-2 from respiratory samples[5,6] and gargle solution.[30] Diverse proteomics sample preparation strategies have been applied for the analysis of SARS-CoV-2-containing samples. In the present study we analyze the inactivation of two human coronaviruses with five different proteomics lysis buffers at two different incubation temperatures. As a control, heat inactivation without lysis buffer was performed.

All inactivation conditions for which the inactivation success could not be confirmed by the absence of a CPE were further investigated. For this purpose, the supernatant of at least one ambiguous sample for each combination of virus, lysis buffer, and temperature were used for three consecutive blind passages in MRC-5 cells. The presence of replication-competent virus particles was controlled by real-time PCR after the third passage. For cells infected with HCoV-OC43 or HCoV-229E, this was done for one GdmCl/*RT* and two urea/*RT* samples or one GdmCl/*RT*, one TFA/*RT*, and one TFA/95 °C sample, respectively. Minor amounts of viral RNA were detected in HCoV-OC43 samples treated with GdmCl and urea ($C_T > 34$) while no viral RNA was detectable in any of the HCoV-229E samples treated with TFA (Table S5).

CONCLUSION

SDS, SDC, and TFA are able to inactivate coronaviruses from cell culture supernatant during a 5 min incubation at room temperature, while GdmCl and urea may lead to incomplete coronavirus inactivation at room temperature. Combining these lysis buffers with heating for 5 min at 95 °C results in complete inactivation but is not recommended for all lysis

https://doi.org/10.1021/acs.jproteome.1c00320
J. Proteome Res. 2021, 20, 4598−4602

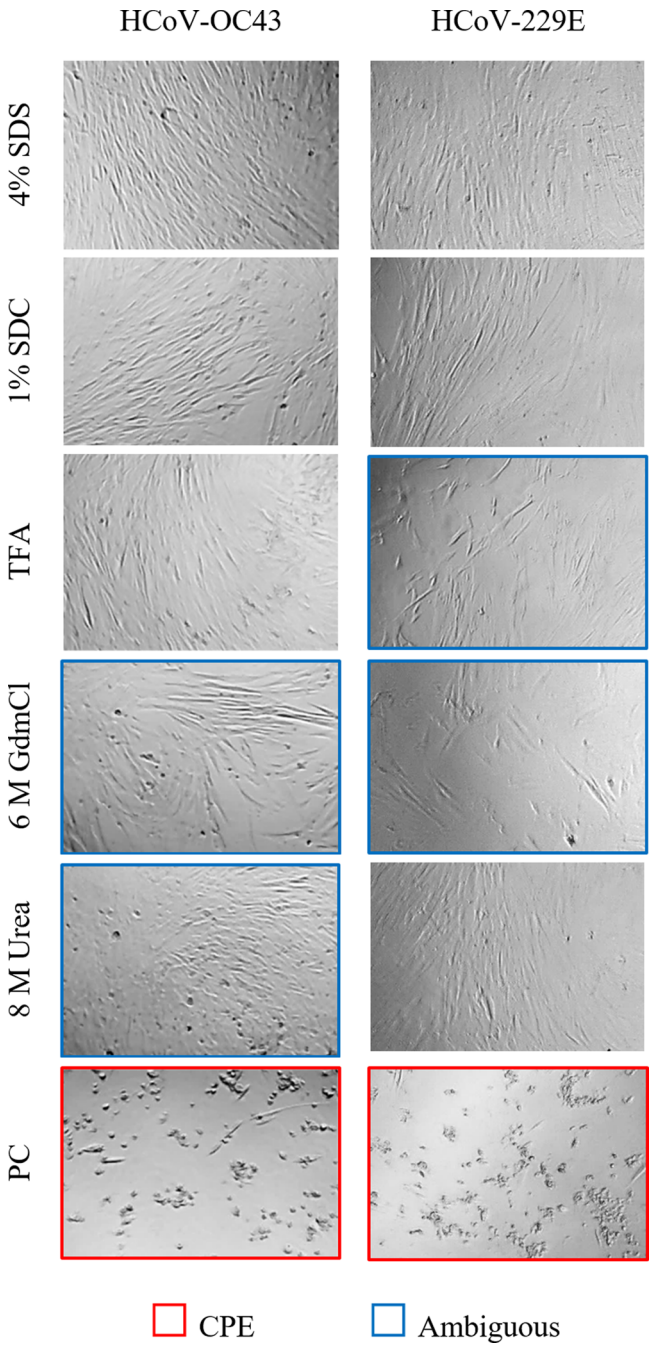


Figure 2. CPE documentation of MRC-5 cells incubated with HCoV-OC43 and HCoV-229E treated with different proteomics lysis buffers. Virus samples were inactivated with different lysis buffers at room temperature (RT) and 95 °C (not shown) and used for inoculation of MRC-5 cells. The cytopathic effect (CPE) was documented for 6 days, and pictures were taken at 4 dpi (HCoV-OC43 samples) or 6 dpi (HCoV-229E samples). CPE (red) and ambiguous morphological structures that were distinct from both the negative and positive control (PC) are indicated. GdmCl: guanidinium chloride. SDC: sodium deoxycholate. SDS: sodium dodecyl sulfate. TFA: trifluoroacetic acid.

buffers. These results demonstrate that lysis buffers should be carefully chosen and tested for inactivation of coronaviruses for proteomics experiments. Finally, it should be noted that in the present study cell culture supernatants were used. However, the inactivation efficiency can be different in other sample materials, like tissue. Hence, other sample material has to be individually evaluated for inactivation success.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.1c00320.

Table S1: Real-time PCR primer and probe sequences. Table S2: Reaction mix for the one-step real-time PCR using AgPath-I One-step RT-PCR Kit. Table S3: Thermal profile for real-time PCR. Table S4: Final viral titers used for inoculation in the inactivation study. Table S5: CT values after lysis buffer inactivation and three consecutive blind passages as determined by real-time PCR. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Marica Grossegesse − *Robert Koch Institute, Centre for Biological Threats and Special Pathogens, 13353 Berlin, Germany;* orcid.org/0000-0002-9369-8203; Phone: +49 30-18754-2140; Email: grossegessem@rki.de

Authors

Paula Leupold − *Robert Koch Institute, Centre for Biological Threats and Special Pathogens, 13353 Berlin, Germany;* Present Address: Freie Universitaet Berlin, Institute of Chemistry and Biochemistry, Thielallee 63, 14195, Berlin, Germany

Joerg Doellinger − *Robert Koch Institute, Centre for Biological Threats and Special Pathogens, 13353 Berlin, Germany; Robert Koch Institute, Centre for Biological Threats and Special Pathogens, 13353 Berlin, Germany;* orcid.org/0000-0001-8309-082X

Lars Schaade − *Robert Koch Institute, 13353 Berlin, Germany*

Andreas Nitsche − *Robert Koch Institute, Centre for Biological Threats and Special Pathogens, 13353 Berlin, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jproteome.1c00320

Author Contributions

#M.G. and P.L. contributed equally.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Melanie Hoffmeister for support with virus propagation and Ursula Erikli for copy-editing. The graphical abstract was created with BioRender.com.

■ REFERENCES

(1) Bojkova, D.; Klann, K.; Koch, B.; Widera, M.; Krause, D.; Ciesek, S.; et al. Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nature* **2020**, *583* (7816), 469−72.

(2) Bouhaddou, M.; Memon, D.; Meyer, B.; White, K. M.; Rezelj, V. V.; Correa, M. M.; et al. The Global Phosphorylation Landscape of SARS-CoV-2 Infection. *Cell* **2020**, *182* (3), 685−712 e19.
(3) Cardozo, K. H. M.; Lebkuchen, A.; Okai, G. G.; Schuch, R. A.; Viana, L. G.; Olive, A. N.; et al. Establishing a mass spectrometry-based system for rapid detection of SARS-CoV-2 in large clinical sample cohorts. *Nat. Commun.* **2020**, *11* (1), 6201.
(4) Grossegesse, M.; Hartkopf, F.; Nitsche, A.; Schaade, L.; Doellinger, J.; Muth, T. Perspective on Proteomics for Virus Detection in Clinical Samples. *J. Proteome Res.* **2020**, *19* (11), 4380−8.
(5) Nikolaev, E. N.; Indeykina, M. I.; Brzhozovskiy, A. G.; Bugrova, A. E.; Kononikhin, A. S.; Starodubtseva, N. L.; et al. Mass-Spectrometric Detection of SARS-CoV-2 Virus in Scrapings of the Epithelium of the Nasopharynx of Infected Patients via Nucleocapsid N Protein. *J. Proteome Res.* **2020**, *19* (11), 4393−4397.
(6) Gouveia, D.; Miotello, G.; Gallais, F.; Gaillard, J. C.; Debroas, S.; Bellanger, L.; et al. Proteotyping SARS-CoV-2 Virus from Nasopharyngeal Swabs: A Proof-of-Concept Focused on a 3 min Mass Spectrometry Window. *J. Proteome Res.* **2020**, *19* (11), 4407−4416.
(7) WHO, Laboratory biosafety guidance related to coronavirus disease 2019 (COVID-19). https://apps.who.int/iris/bitstream/handle/10665/331138/WHO-WPE-GIH-2020.1-eng.pdf. 2020.
(8) Kampf, G.; Voss, A.; Scheithauer, S. Inactivation of coronaviruses by heat. *J. Hosp Infect.* **2020**, *105* (2), 348−9.
(9) Xiling, G.; Yin, C.; Ling, W.; Xiaosong, W.; Jingjing, F.; Fang, L.; et al. In vitro inactivation of SARS-CoV-2 by commonly used disinfection products and methods. *Sci. Rep.* **2021**, *11* (1), 2418.
(10) Wisniewski, J. R.; Zougman, A.; Nagaraj, N.; Mann, M. Universal sample preparation method for proteome analysis. *Nat. Methods* **2009**, *6* (5), 359−62.
(11) Pasing, Y.; Colnoe, S.; Hansen, T. Proteomics of hydrophobic samples: Fast, robust and low-cost workflows for clinical approaches. *Proteomics* **2017**, *17* (6), 1500462.
(12) Shu, T.; Ning, W.; Wu, D.; Xu, J.; Han, Q.; Huang, M.; et al. Plasma Proteomics Identify Biomarkers and Pathogenesis of COVID-19. *Immunity* **2020**, *53* (5), 1108−22 e5.
(13) Doellinger, J.; Schneider, A.; Hoeller, M.; Lasch, P. Sample Preparation by Easy Extraction and Digestion (SPEED) - A Universal, Rapid, and Detergent-free Protocol for Proteomics Based on Acid Extraction. *Mol. Cell Proteomics.* **2020**, *19* (1), 209−22.
(14) Grossegesse, M.; Nitsche, A.; Schaade, L.; Doellinger, J. Application of spectral library prediction for parallel reaction monitoring of viral peptides. *Proteomics* **2021**, *21*, No. e2000226.
(15) Soday, L.; Lu, Y.; Albarnaz, J. D.; Davies, C. T. R.; Antrobus, R.; Smith, G. L.; et al. Quantitative Temporal Proteomic Analysis of Vaccinia Virus Infection Reveals Regulation of Histone Deacetylases by an Interferon Antagonist. *Cell Rep.* **2019**, *27* (6), 1920−33 e7.
(16) Bian, Y.; Zheng, R.; Bayer, F. P.; Wong, C.; Chang, Y. C.; Meng, C.; et al. Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC-MS/MS. *Nat. Commun.* **2020**, *11* (1), 157.
(17) Reed, L. J.; Muench, H. A simple method of estimating fifty-percent endpoints. *Am. J. Epidemiol.* **1938**, *27* (3), 493−497.
(18) Coves-Datson, E. M.; King, S. R.; Legendre, M.; Gupta, A.; Chan, S. M.; Gitlin, E.; et al. A molecularly engineered antiviral banana lectin inhibits fusion and is efficacious against influenza virus infection in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (4), 2122−32.
(19) Meyers, C.; Kass, R.; Goldenberg, D.; Milici, J.; Alam, S.; Robison, R. Ethanol and isopropanol inactivation of human coronavirus on hard surfaces. *J. Hosp Infect.* **2021**, *107*, 45−9.
(20) Hulkower, R. L.; Casanova, L. M.; Rutala, W. A.; Weber, D. J.; Sobsey, M. D. Inactivation of surrogate coronaviruses on hard surfaces by health care germicides. *Am. J. Infect. Control* **2011**, *39* (5), 401−7.
(21) Rabenau, H. F.; Schwebke, I.; Blumel, J.; Eggers, M.; Glebe, D.; Rapp, I.; et al. [Guideline of the German Association for the Control of Viral Diseases (DVV) eV and the Robert Koch Institute (RKI) for testing chemical disinfectants for effectiveness against viruses in human medicine. Version of 1 December, 2014]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* **2015**, *58* (4−5), 493−504.
(22) Barrow, K. A.; Rich, L. M.; Vanderwall, E. R.; Reeves, S. R.; Rathe, J. A.; White, M. P. Inactivation of Material from SARS-CoV-2-Infected Primary Airway Epithelial Cell Cultures. *Methods Protoc.* **2021**, *4* (1), 7.
(23) Patterson, E. I.; Prince, T.; Anderson, E. R.; Casas-Sanchez, A.; Smith, S. L.; Cansado-Utrilla, C.; et al. Methods of Inactivation of SARS-CoV-2 for Downstream Biological Assays. *J. Infect. Dis.* **2020**, *222* (9), 1462−7.
(24) Welch, S. R.; Davies, K. A.; Buczkowski, H.; Hettiarachchi, N.; Green, N.; Arnold, U., et al. Analysis of Inactivation of SARS-CoV-2 by Specimen Transport Media, Nucleic Acid Extraction Reagents, Detergents, and Fixatives. *J. Clin. Microbiol.* **2020**; *58* (11). DOI: 10.1128/JCM.01713-20.
(25) Kollipara, L.; Zahedi, R. P. Protein carbamylation: in vivo modification or in vitro artefact? *Proteomics* **2013**, *13* (6), 941−4.
(26) Gordon, D. E.; Hiatt, J.; Bouhaddou, M.; Rezelj, V. V.; Ulferts, S.; Braberg, H.; et al. Comparative host-coronavirus protein interaction networks reveal pan-viral disease mechanisms. *Science* **2020**, *370* (6521), No. eabe9403.
(27) Li, J.; Guo, M.; Tian, X.; Wang, X.; Yang, X.; Wu, P.; et al. Virus-Host Interactome and Proteomic Survey Reveal Potential Virulence Factors Influencing SARS-CoV-2 Pathogenesis. *Med. (N Y).* **2021**, *2* (1), 99−112 e7.
(28) Zhang, H.; Zheng, H.; Zhu, J.; Dong, Q.; Wang, J.; Fan, H. Ubiquitin-Modified Proteome of SARS-CoV-2-Infected Host Cells Reveals Insights into Virus-Host Interaction and Pathogenesis. *J. Proteome Res.* **2021**, *20*, 2224.
(29) Park, J.; Kim, H.; Kim, S. Y.; Kim, Y.; Lee, J. S.; Dan, K.; et al. In-depth blood proteome profiling analysis revealed distinct functional characteristics of plasma proteins between severe and non-severe COVID-19 patients. *Sci. Rep.* **2020**, *10* (1), 22418.
(30) Ihling, C.; Tänzler, D.; Hagemann, S.; Kehlen, A.; Hüttelmaier, S.; Arlt, C.; et al. Mass Spectrometric Identification of SARS-CoV-2 Proteins from Gargle Solution Samples of COVID-19 Patients. *J. Proteome Res.* **2020**, *19* (11), 4389−4392.

https://doi.org/10.1021/acs.jproteome.1c00320
J. Proteome Res. 2021, 20, 4598−4602