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Article

SARS-CoV-2 inactivation by a panel of commercialized buffers and by traditional protocols (heat or SDS)

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Abstract:

Following the rapid spread of COVID-19 across the globe, the intense response that was demanded of diagnostic centers and research laboratories prompted the use of numerous products and protocols for the management of SARS-CoV-2 specimens. In these settings, proper handling of such infectious specimen is necessary to ensure the safety of personnel and to reduce the risk of active transmission. Our aim was to evaluate the inactivation efficacy of different inactivating methods, notably from commercial lysis buffers available in diagnostic kits. Heat and sodium dodecyl sulfate detergent were also included in our investigations. A cell culture-based assay was used, and supported by molecular qRT-PCR detection, to show *in vitro* infectivity reduction after treatment. Overall, all the investigated methods were successful in inactivating SARS-CoV-2. Ten minutes of contact with the commercial buffers completely stopped *in vitro* SARS-CoV-2 infectivity. Fifteen minutes at 68°C and 30 minutes at 56°C as well as 30 minutes with sodium dodecyl sulfate detergent at 2, 1, 0.5, and 0.1% yielded similar results. These findings demonstrate the reliability of these protocols with regards to biosafety. Inactivation by heat and sodium dodecyl sulfate detergent are rather simple and can be readily available methods for rendering an infectious SARS-CoV-2 specimen inactive, especially in settings where commercial buffers are not available.

Keywords: SARS-CoV-2; inactivation; commercial buffer; heat; SDS

1. Introduction

Following the emergence of the first cases of COVID-19 (coronavirus disease 2019) in December 2019 detected by the Chinese health authorities, the causative agent SARS-CoV-2 (severe acute respiratory coronavirus 2) was lab-isolated by January 7, and the full genome sequence was published online, freely available, by January 12, 2020 [1,2]. The swift actions of the Chinese health authorities allowed for an equally quick response, albeit limited at the time, by health authorities around the world to deploy laboratory diagnostics for this alarming global health crisis [3,4]. Government response tactics to population testing varied greatly both at the early timeline of local transmission in the first quarter of 2020 and throughout the different peaks of regional or national epidemiological dynamics [5–7]. For SARS-CoV-2 diagnosis, reliable and sensitive real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) assays was and is still an essential tool to curb the evolution of the COVID-19 pandemic [8].

The widespread dissemination of the virus across the globe and its overbearing, multi-faceted impact on society have pushed efforts for testing, mitigation, and therapeutics to increase exponentially both in the private and public sectors. With the unprecedented high demand of diagnostics tests performed around the world, the question of

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handling and processing such infectious samples remains relevant. Inactivation protocols against viruses have been regularly evaluated in the past, including with heat against influenza [29,30] and SARS-CoV 2003 [31] viruses, as well as with detergents against enveloped herpes simplex and human immunodeficiency virus [32]. Indeed, with over 426 million global COVID-19 cases diagnosed by mid-February 2022 [9], public health laboratories have commissioned incremental changes to logistics processing and staff organization. Furthermore, fundamental research on SARS-CoV-2, classified by the US CDC [10] and WHO [11] as a Risk Group 3 pathogen, requires appropriate bio-safety laboratory equipment and protective measures.

For these reasons, the objective of our work was to evaluate the inactivation efficacy of simple chemical and physical methods to secure the safety of personnel handling infectious SARS-CoV-2 specimens.

2. Materials and Methods

A SARS-CoV-2 isolate belonging to the 20C clade (GISAID Accession number EPI_ISL_640002) was obtained by the French National Reference Centre for Respiratory Viruses (Lyon, FR) and propagated on Vero cells (ATCC©, CCL-81) with EMEM culture media, supplemented with 2% Penicillin Streptomycin + 1% L-Glutamine + 2% fetal bovine serum.

The commercial buffers from diagnostic kits tested included the Bioer preservation buffer (Bioer, CN), Cobas® lysis buffer (Roche, DE), Cobas® viral transport medium (Roche, DE), MGI viral transport medium (MGI Tech Co. Ltd., CN), NucliSENS® EMAG® lysis buffer (bioMérieux, FR), Panther Fusion™ lysis buffer (Hologic®, USA), and SunTrine® viral transport medium (SunTrine® Biotechnologies, CN). Heat inactivation was carried out with a dry-heat oven, and sodium dodecyl sulfate (SDS, CAS n°151-21-3) was used at final concentrations of 2, 1, 0.5, and 0.1%.

For commercial buffers, 100 uL of SARS-CoV-2 culture sample were added to 900 uL commercial buffer and then incubated at room temperature for 10 minutes. For the heat protocols, 100 uL of culture sample was added to 900 uL of supplemented culture media and incubated at 56°C for 30 minutes or 68°C for 15 minutes. For the SDS protocols, 100 uL of SARS-CoV-2 culture sample were added to 900 uL of supplemented culture media with the appropriate quantity of SDS for final concentrations described above and then incubated at room temperature for 30 minutes.

Once the contact period completed, each inactivation condition was diluted through 10-fold serial dilutions (up to 10^{-6}) in supplemented EMEM culture media and then inoculated in triplicate on confluent Vero cells seeded in 96 well plates. To account for the eventual cytotoxic property of the chemical agents, control wells were prepared for all inactivation conditions in the absence of virus. The plates were incubated for 96 hours at 36°C under 5% CO_2. Cytotoxicity and virus growth were monitored by optical microscopy. To confirm the observation of cytopathic effects (CPE), the supernatant of each condition was sampled after the contact period (D0) and again after 96 hours of incubation (D4) for RNA extraction and qRT-PCR detection with the TaqPath COVID-19 CE-IVD kit (ORF1ab target gene; ThermoFisher Scientific) on a QuantStudio 5 System (Applied Biosystems) [12].

Infectious titers were calculated with the Spearman-Karber method [13]. The analysis of SARS-CoV-2 inactivation was based on recommendations of the European norm NF EN 14476-A2 [14], where the difference between infectious titers of untreated and treated conditions constitutes the $\log_{10}$ reduction value (LRV).

3. Results

3.1. Untreated virus control

The untreated SARS-CoV-2 control yielded typical CPE, defined by morphological changes such as rounding of cells and lysis of the cell monolayer (Figure 1). This SARS-

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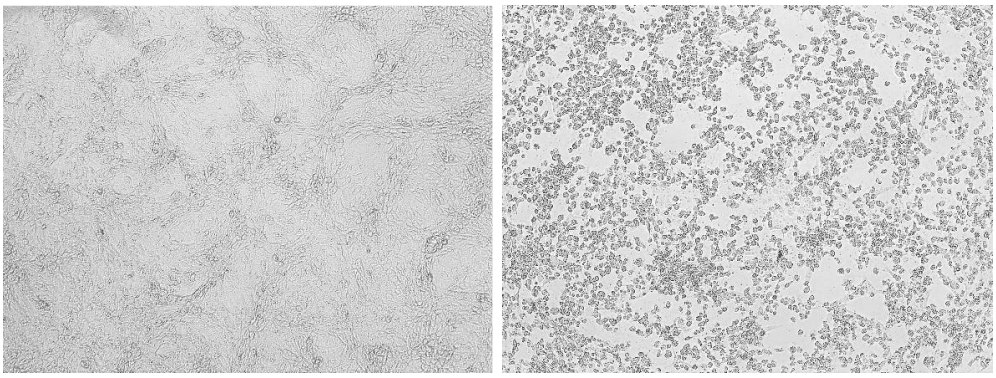
CoV-2 cell culture passage resulted in an average infectious titer of 5.55 log_{10} $TCID_{50}$/mL (Table 1). Semi-quantitative qRT-PCR data supported these findings, with active virus genome replication detected for all CPE-positive wells (Supplemental Data 1).



(a) (b)

Figure 1. Optical microscopy photos after 96 hours of incubation of (**a**) an intact Vero cell monolayer and (**b**) a damaged Vero cell monolayer with rounded cells due to active SARS-CoV-2 replication and cell lysis.

3.2. Commerical buffers

Cytotoxicity was observed for all commercial buffers tested at the more concentrated dilutions. Nevertheless, the 10-fold serial dilutions provided a cytotoxic limit from which cytotoxicity was no longer observed. No CPE were observed for any commercial buffer (Table 1). Semi-quantitative qRT-PCR data supported these findings, with no active genome replication detected for CPE-negative wells (Supplemental Data 1). All commercial buffers therefore yielded a >5 log_{10} reduction of active SARS-CoV-2 replication.

3.3. Heat

No CPE were observed for conditions treated with either heat protocols (Table 1). Semi-quantitative qRT-PCR data supported these findings. Both heat protocols therefore also yielded a >5 log_{10} reduction of active SARS-CoV-2 replication.

3.4. Sodium dodecyl sulfate detergent

Cytotoxicity was observed for all initial SDS concentrations, but the 10-fold serial dilutions provided a cytotoxic limit from which cytotoxicity was no longer observed. No CPE were observed for any SDS concentration (Table 1). As with the previous inactivation methods, semi-quantitative qRT-PCR data supported these findings, with no active genome replication detected for CPE-negative wells. All SDS concentrations yielded a >5 log_{10} reduction of active virus replication.

Table 1. SARS-CoV-2 inactivation by commercial buffers, heat, and SDS detergent observed by the infectious titer after 96 hours of incubation post-treatment. $TCID_{50}$, median tissue culture infectious dose; LRV, log_{10} reduction values.

Inactivation method	**Log_{10} $TCID_{50}$/mL**	**LRV**
Virus control (untreated)	5.55	
Commercial diagnostic buffers (10 min)		
Bioer preservation buffer	0	>5
Cobas® lysis buffer	0	>5
Cobas® viral transport medium	0	>5
MGI viral transport medium	0	>5
NucliSENS® EMAG® lysis buffer	0	>5

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Panther Fusion™ lysis buffer	0	>5
SunTrine® viral transport medium	0	>5
Heat		
56°C (30 min)	0	>5
68°C (15 min)	0	>5
SDS detergent (30 min)		
2.0%	0	>5
1.0%	0	>5
0.5%	0	>5
0.1%	0	>5

4. Discussion

According to the European NF EN 14476-A2 and WHO recommendations on robust and reliable viral safety, the standard acceptance criteria for virucidal substances is its ability to remove or inactivate 4 $\log_{10}$ or more amounts of virus [14,15]. Hence, our data demonstrate conclusive SARS-CoV-2 inactivation efficacy for all investigated methods, with greater than 5 $\log_{10}$ infectious titer reduction. Inactivation efficacy has been described with past studies on heat inactivation against influenza [16,17] and SARS-CoV 2003 [18] viruses as well as on detergent inactivation against enveloped herpes simplex and human immunodeficiency virus [19]. In the present study, we find similar findings for heat and detergent inactivation against SARS-CoV-2 [20–22] but with more SDS concentrations tested and the addition of commercial buffers widely used in France and in Europe during the pandemic.

In vitro virus culture assays based on the observation of cytopathic effects rely on the ability of a given virus to produce a cellulolytic effect on the susceptible and permissive cell line being infected. However, not all viruses that are culture-positive produce CPE on a chosen cell line, and other methods of detection are therefore necessary. In the case of SARS-CoV-2, the Vero cell line is the most widely used non-human cell line, as has been common practice for isolation and propagation of other coronaviruses [23]. In addition to reliable CPE observation, culture wells were further screened for viral load evolution via semi-quantitative qRT-PCR. This additional technique allows for the confirmation of CPE-positive wells, with an exponential increase in viral load after 96 hours, and of CPE-negative wells, with a stable or even decreased viral load after 96 hours. Together, both datatypes demonstrated consistently congruent interpretations for the present study. This applies more generally to virus isolates that are propagated on and adapted to specific cell lines.

Nevertheless, it is known that in some cases, CPE and PCR data interpretation are divergent. This is most commonly found *in vivo*, such as in the case of using recovering COVID-19 patient samples during the later phases of infection or persistent cases, where results are PCR-positive but CPE-negative in cell culture [12,24]. In fact, CPE-negative samples are often associated with neutralizing antibodies from patient sera, indicating that the virus or fragments of the virus are present but no longer infectious [12]. These interpretations are pertinent in the context of clinical patient management and have indeed influenced COVID-19 health policies but are outside the scope of our current objective in testing the ability to inactivate infectious SARS-CoV-2.Experimentally, a culture isolate is less variable than a clinical sample, thus more compatible for intrinsic viral interpretation.

The qRT-PCR data revealed some culture assay conditions with undetectable levels of the targeted viral genome. For COVID-19 diagnosis, this could be concerning if interpreted as a false negative result. However, it is important to note that the condition closest to a diagnostic setting is the dilution 10^{-1} and our data assuredly demonstrates that all inactivation methods retained PCR sensitivity with PCR cycle thresholds comparable to the untreated virus control irrespective of the time point. This shows that the tested methods in the present study do not impact their use for diagnostics.

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In wake of the global health crisis, many forms of biological samples potentially carrying infectious SARS-CoV-2 need to be handled, such as culture isolates and more complex specimen including sputum, plasma, and stool, etc. [25–27]. The methods to inactivate these different samples can vary greatly, but the most common are heat and detergents [20,21,28–31]. Numerous studies have even described SARS-CoV-2 inactivation efficacy with less traditional protocols including ophthalmic solutions, repurposed therapeutic agents or ultraviolet C irradiation [28,32–34]. While these efforts contribute to our growing knowledge on SARS-CoV-2, not all protocols may be readily available options to many medical or non-medical institutions and facilities. Furthermore, designed for their practicality and having been essential in the fight against COVID-19, commercial diagnostic kits saw their availability fluctuate due to high global demand and supply chain challenges during the pandemic. Heat and SDS seem to be easily accessible and simple procedures for SARS-CoV-2 inactivation, with our data showing efficacy by SDS alone at concentrations as low as 0.1% when others reported SDS at higher concentrations and/or in combination with other agents [22,35,36].

All described methods may also impact the integrity of the virus genome and protein structures and therefore constitutes another factor to consider when adopting an appropriate inactivation protocol. For example, genome instability with qRT-PCR detection has been reported, especially with regards to high heat inactivation showing an inappropriate protocol at 92°C [21,37]. Other methods employing gamma irradiation and β-propiolactone, aim to conserve the viral proteins and have specifically been studied for the development of inactivated vaccines [38,39].

5. Conclusions

Overall, our study demonstrates the effective virus inactivation of active SARS-CoV-2 replication by various commercial buffers, heat, and SDS detergent. Commercial lysis and transport buffers have the potency to inactivate the virus alone, so subsequent heat or SDS inactivation of SARS-CoV-2 samples collected with these solutions is not necessary for diagnostics. To conduct SARS-CoV-2 research at lower bio-containment levels, 56°C for 30 minutes, 68°C for 15 minutes, and SDS as low as 0.1% are effective methods of inactivation, but the choice of protocol should always be in adequation with the downstream biological process and is still contingent on respecting maximum risk management.

Supplementary Materials: Supplementary Data 1

Author Contributions: Conceptualization, A.G., F.M., and M.B.; methodology, A.G., F.M., and M.B.; validation, A.G., F.M. and M.B.; formal analysis, A.G. and G.Q.; investigation, A.G. and G.Q.; resources, M.V.; data curation, A.G., A.O. and G.Q.; writing—original draft preparation, A.G. and G.Q.; writing—review and editing, A.G., B.L., E.F., G.B., G.Q., F.M., M.B., M.V., and V.E.; supervision, E.F., F.M. and B.L.; All authors have read and agreed to the published version of the manuscript.

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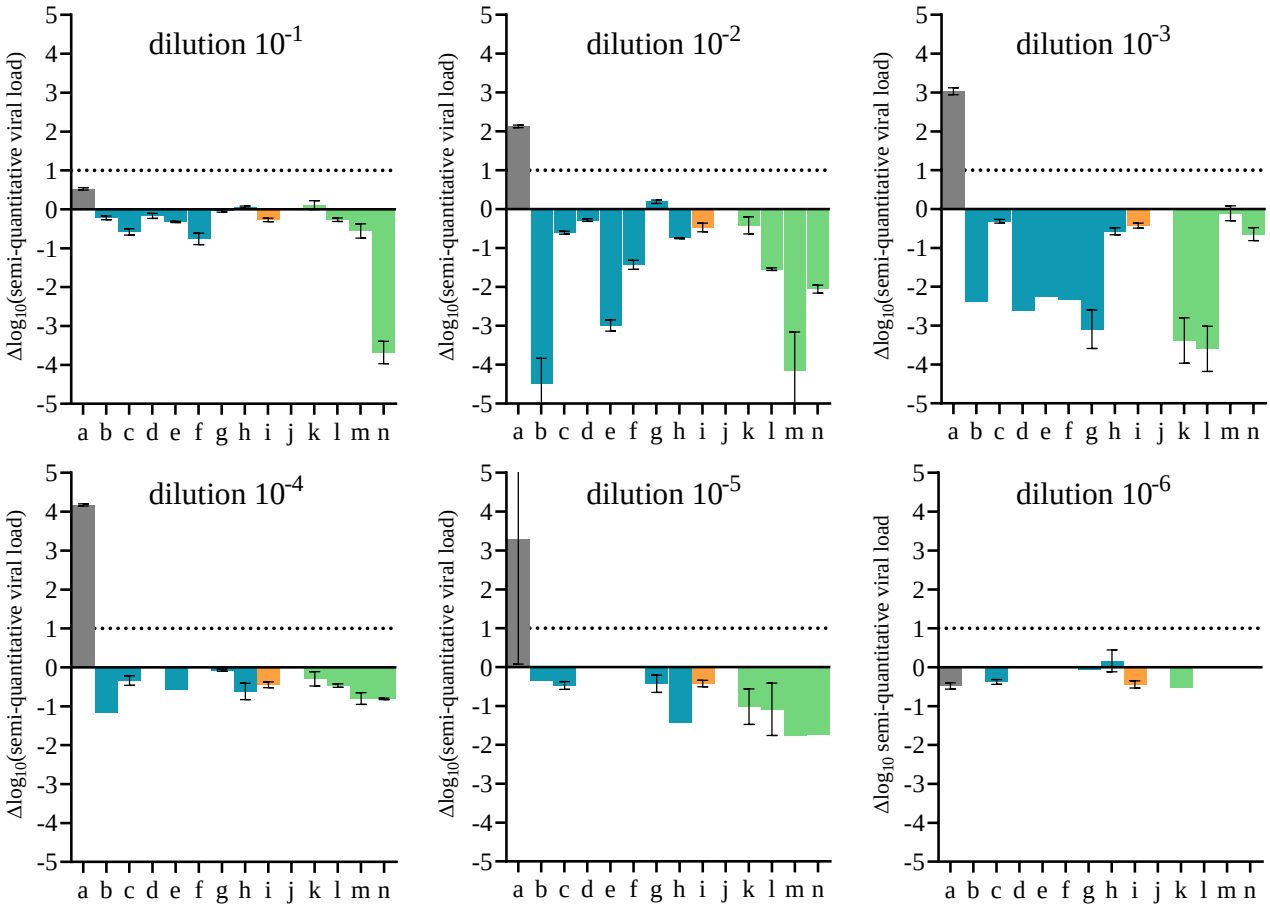

a) Virus control

commercial diagnostic buffers (10 min)
b) Bioer preservation buffer
c) Cobas® lysis buffer
d) Cobas® viral transport medium
e) MGI viral transport medium
f) NucliSENS® EMAG® lysis buffer
g) Panther Fusion™ lysis buffer
h) SunTrine® viral transport medium

heat
i) 56°C (30 min)
j) 68°C (15 min)

sodium dodecyl sulfate (30 min)
k) SDS 2.0%
l) SDS 1.0%
m) SDS 0.5%
n) SDS 0.1%

Supplementary Data 1. The differential semi-quantitative SARS-CoV-2 viral load, expressed as $\Delta\log_{10}$, detected from a 96-hour interval after performing inactivation protocols. Virus supernatants were sampled immediately after each inactivation protocol was completed and again after 96 hours of incubation on Vero cells in supplemented culture media at 36°C under 5% CO_2. SARS-CoV-2 RNA was extracted from the supernatants and detected by semi-quantitative qRT-PCR. Represented above are all tested serial dilutions (10^{-1} to 10^{-6}). The horizontal dotted line at 1 $\Delta\log_{10}$ is an arbitrary threshold signifying a significant increase in detected viral load (active SARS-CoV-2 replication). Null and negative $\Delta\log_{10}$ values both represent inactive SARS-CoV-2, with implied RNA degradation for the latter. For the dilution 10^{-1}, the $\Delta\log_{10}$ of the untreated virus control is counterintuitively low because of the amplification plateau limit inherent to the qRT-PCR technique; the initial quantity of virus inactivated and the quantity of virus after 96 hours of incubation were both simply at levels near or above this quantification plateau.